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PHYSIOLOGICAL STUDIES WITH THE NITROGEN-FIXING ALGA, NOSTOC MUSCORUM

FRANKLIN E. ALLISON, SAM R. HOOVER, AND HERMAN J. MORRIS

(WITH NINE FIGURES)

Introduction

The ability of certain species of blue-green algae to utilize free nitrogen when grown in pure culture was not conclusively proved until the year 1928, although much early work with mixed cultures of algae and bacteria had suggested this possibility. DREWES (15) grew *Anabaena variabilis*, *Nostoc punctiforme*, and a second species of *Anabaena* in pure culture on a nitrogen-free medium and obtained nitrogen fixation. ALLISON and MORRIS (3, 4) also isolated a blue-green alga from the soil and demonstrated its nitrogen-fixing ability. At the time it was thought to be a species of *Anabaena*, but later work has shown that it is a *Nostoc*. Further studies with this organism were reported in brief recently (1), and the present paper gives additional data with regard to the conditions which favor nitrogen fixation by this common soil organism. COPELAND (14) reported that certain, but not all, thermal strains of the Myxophyceae, including strains of *Oscillatoria princeps*, *O. formosa*, *Spirulina labyrinthiformis*, and *Phormidium laminosum*, isolated from nitrogen-deficient warm springs, utilize atmospheric nitrogen.

Some of the endophytic blue-green algae which live in symbiosis with higher green plants (*Blasia*, *Cavicularia*, *Anthoceros*, *Azolla*, *Cycas*, *Gunnera*, and *Encephalartos*) have also been shown to be able

to secure their nitrogen from the air. VOUK and WELLISCH (34) discuss the available information on this subject. MOLISCH (25) obtained bacteria-free cultures of *Nostoc* from *Blasia* and *Cavicularia* and demonstrated that they grew as well on nitrogen-free media as in the presence of potassium nitrate, but did not report nitrogen analyses. VOUK and WELLISCH likewise obtained good growths of *Nostoc* from *Anthoceros* and *Blasia*, and of *Anabaena azollae* on nitrogen-free media. WINTER (35) also has recently shown that *Nostoc punctiforme*, isolated from five host plants and grown in pure culture (two out of the five cultures), fixed up to 1.95 mg. nitrogen per 50 cc. of culture solution. Fixation did not occur in the absence of a suitable sugar source.

Observations

DESCRIPTION OF ORGANISM

Extensive microscopic studies, carried out in part in cooperation with Dr. FRANCIS DROUET of the University of Missouri, have shown that the characteristics of the organism used in these investigations agree in every essential respect with those of *Nostoc muscorum* Ag. ex Born et Flah. The identification of the organism proved rather difficult because it grows somewhat differently in artificial culture solutions than in nature. In soil where the moisture is limited, the colonies may contain few trichomes (filaments), whereas in solution cultures the organism sometimes continues to grow for long periods, largely as trichomes, often lying nearly parallel, as in the case of *Anabaena*.

The spores are usually somewhat smaller than most of the descriptions of the organism specify, but it is very probable that the smaller spore size is the more common, judging by the observations of BRISTOL (7), LOWE and MOYSE (23), and ourselves. Wide variation in spore size is at least a well recognized characteristic (6, 19, 33). The colonies, as we observed them on soil kept in Erlenmeyer flasks plugged with cotton, were less gelatinous and more inclined to spread in thin surface films than other workers have reported. This was undoubtedly due to the more abundant supply of moisture in the soil and air under our conditions. Attempts to secure strains of

Nostoc muscorum from other workers for direct comparisons of growth characteristics were unsuccessful.

A description of the organism as we found it, grown chiefly in liquid and agar cultures using inorganic, sugar-free media, is as follows. A few cultures were also grown on soil.

PLANT MASS.—On soil the colonies are gelatinous-membranaceous, nearly round at first, commonly 1–5 cm. in diameter but expanding irregularly, especially in the presence of an abundance of moisture, and are dark olive green to yellowish brown. On agar the colonies usually spread more rapidly to form a thin surface layer. In liquid media thin, widely spreading light or dark green films form, which if undisturbed remain largely at the surface, being supported in part by the bubbles of gas given off during photosynthesis.

TRICHOMES.—In young cultures the trichomes or filaments are frequently motile, nearly straight, and may lie more or less parallel; usually flexuous and densely entangled later. Sheaths are plainly visible surrounding the older forms. In old cultures these filaments often break up, leaving the individual cells essentially free but held together in jelly-like masses. Motile trichomes or hormogones may form as the result of the repeated division of these individual cells, by the breaking of the older trichomes at the heterocysts or elsewhere, or by spore germination.

CELLS.—Vegetative cells are of varying shapes, spherical, barrel-shaped, cylindrical, or quadrate, 2.5–4 μ broad, 3.5–5 μ long. Young cells are usually barrel-shaped but older cells are commonly round or oval.

HETEROCYSTS.—Formed abundantly, almost round and when mature 5–7 μ in diameter. Both the intercalary and terminal types are common, usually one to three per trichome but occasionally as many as twenty being observed. The intercalary type is almost round and when mature 5–7 μ in diameter. The true terminal heterocysts are formed terminally, are somewhat pointed, and have one polar nodule instead of two as in the intercalary type.

SPORES.—Often formed abundantly in older cultures, especially where these are allowed to dry out slowly. Develop in catenate series between heterocysts. In some cases nearly all vegetative cells may be converted into spores. Usually oval or nearly spherical but

may be somewhat angular. Size 4–6 μ wide and 4–10 μ long. The more common size is 4–5 μ wide and 6–7 μ long. Walls smooth. Color yellow or yellowish brown when mature.

HABITAT.—Isolated from the surface of moist shaded soil and later from a small pool of water in the District of Columbia. Preserved specimens of this organism will be placed in the U.S. National Herbarium, the New York Botanical Garden, and the Farlow Herbarium at Harvard University.

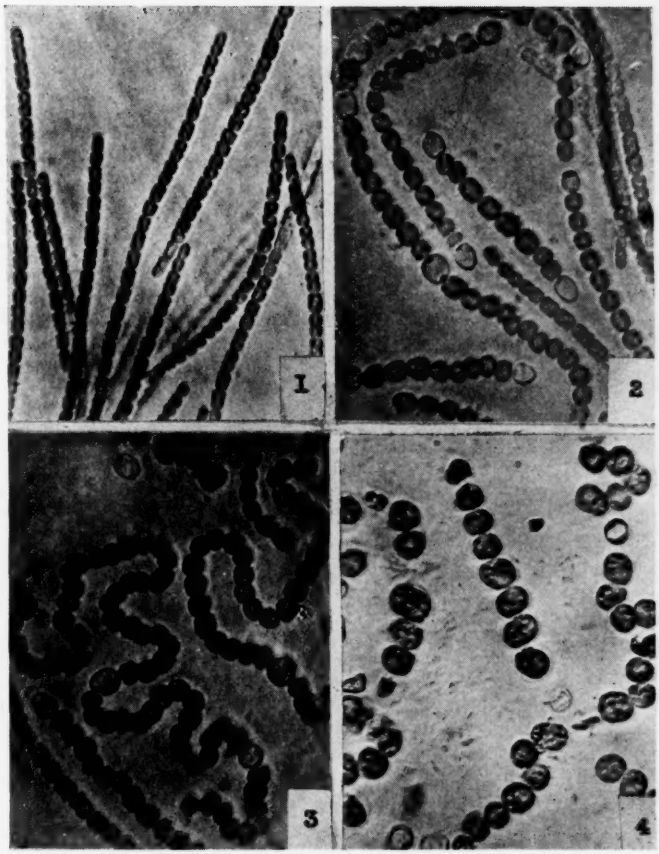
Photomicrographs of the organism are shown in figures 1–8.

METHODS

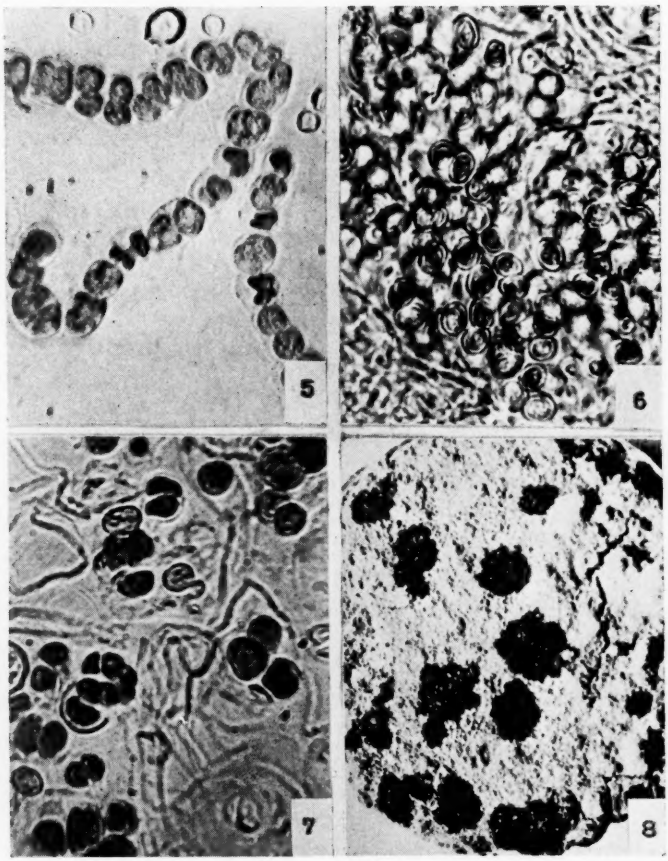
The experiments were carried out over a period of about six years, using a variety of media and growth conditions. The primary purpose of the work was, in fact, to determine the optimum conditions for growth and nitrogen fixation and some of the factors which affect the rate of fixation. Only representative experiments of a large number carried out are reported here. The media used in each experiment, as well as the specific growth conditions, are given in connection with each table, since uniform experimental conditions were not used throughout the work. Artificial light, supplied by a bank of 60 to 100 watt Mazda lamps, was used as the light source. The daily period of illumination varied from 12 to 24 hours.

Erlenmeyer flasks of 250 cc. capacity, fitted with cotton plugs and containing 100 cc. of media, were used in growing the unaerated cultures; 500 cc. pyrex gas washing bottles, ordinarily containing 100 cc. of media, were used for the aerated cultures. Aeration was accomplished by passing compressed air, containing the desired percentage of CO_2, through concentrated H_2SO_4 to remove any ammonia or oxides of nitrogen and then through a solution of $HgCl_2$. The culture vessels were usually maintained at a temperature of 24°–27° C. by keeping them immersed in a water bath to a depth of about 1 inch.

All media were sterilized in the autoclave for a period of 15 to 20 minutes at 15 pounds' pressure, except where glucose was present, in which case the time was 10 minutes at 10 pounds' pressure. All the usual precautions against contaminations were exercised. Inoculations were made from a young culture, grown on a sugar medium in



FIGS. 1–4.—*Nostoc muscorum* Ag., photomicrographs of living unstained cells from liquid inorganic nitrogen-free media: Fig. 1, young actively growing, motile trichomes, 20 days old. Fig. 2, trichomes that have come to rest and formed terminal and intercalary heterocysts; motile trichomes (without heterocysts) also present; age of culture 27 days. Fig. 3, stage in growth of organism just prior to breaking of trichomes at heterocysts to form motile hormogones; continued cell division of non-motile trichomes, such as shown in fig. 2, produces these contorted trichomes; culture 27 days old. Fig. 4, old trichomes with individual cells essentially free but held together in jelly-like masses, a few starting to germinate; culture 10 months old. ×780.



FIGS. 5–8.—*Nostoc muscorum:* Fig. 5, cell division in old vegetative cells seen occasionally; each cell may develop into a trichome; inorganic, nitrogen-free medium; culture 10 months old. ×780. Fig. 6, spores thoroughly desiccated, shown imbedded in mass of fungus filaments. ×780. Fig. 7, germinating spores (as in fig. 6) 10 days after addition to fresh medium. ×780. Fig. 8, colonies on soil. Approximately normal size.

order the more readily to detect contaminants. Usually a very small quantity of young trichomes from a culture, grown in a liquid medium, was transferred by means of a sterile wire. This method is not entirely satisfactory because it is impossible to add the same number of cells to the various flasks. Inoculations may also be made by means of a pipette if a culture having an abundance of young motile organisms is used. An attempt was made to use this system, but it was not very satisfactory because motile trichomes commonly become non-motile and form films and later gelatinous masses very quickly.

All nitrogen analyses were made by the macro-Kjeldahl method, using H_2SO_4, $CuSO_4$, and K_2SO_4 in the digestion flasks. The algal cells and medium in which they had grown were analyzed together unless otherwise stated.

Media

Nostoc muscorum was found to grow readily upon a wide variety of media such as are commonly used in growing *Azotobacter*, *Rhizobium*, and green algae; yet it is not always easy to grow it in the laboratory for long periods so as to obtain large masses of the organism, unless its growth requirements are understood. Very slow growth or death may result from a variety of causes, but numerous experiments have shown that the development of an unfavorable pH is usually the primary factor. The shift may be toward either the acid or alkaline side, depending upon the medium used and the growth conditions. These will be discussed later.

Tables 1–8 give in part the results of the studies on media, but for the sake of brevity they will not be discussed individually in this connection. All the results, together with numerous observations and preliminary experiments not reported here, are summarized later.

Aerated cultures.—The following medium was found to be very suitable for the growth of cultures aerated with air containing 1 per cent CO_2: K_2HPO_4, 0.5 gm.; $MgSO_4 \cdot 7H_2O$, 0.2 gm.; NaCl, 0.2 gm.; $CaSO_4 \cdot 2H_2O$, 0.1 gm.; $FeCl_3 \cdot 6H_2O$, 0.005 gm.; $CaCO_3$, 1–5 gm.; H_2O, 1000 cc. Natural humic acid or synthetic iron humate (**2, 11, 12**) at the rate of 5 p.p.m. may sometimes be substituted with ad-

vantage for the ferric chloride. The NaCl is probably not essential. This medium usually has a pH of 8 or higher after autoclaving, but when aerated with 1 per cent CO_2 in air the pH drops to near 7.

TABLE 1

EFFECT OF NATURAL HUMIC ACID ON NITROGEN FIXATION

Treatment	Nitrogen added per 100 cc. (mg.)	First experiment* (unaerated)		Second experiment (aerated 1% CO_2 in air)		
		Total nitrogen (mg.)	Average nitrogen fixed (mg.)	Total nitrogen (mg.)	Average nitrogen fixed (mg.)	Final pH
Check (basal medium only)	0	8.45 9.68	9.07	8.02 8.70	8.36	7.2 7.6
Asparagin	5	14.27 13.42	8.85	9.92 9.67	4.80	7.2 7.3
KNO_3	5	.....	.....	9.40 9.43	4.42	7.7 7.7
Humic acid, 10 p.p.m.	0.04	14.23 15.69	14.92	.....	.....	.....
Humic acid, 20 p.p.m.	0.1	.....	.....	17.40 12.07	14.64	7.0 7.1
Humic acid, 100 p.p.m.	0.42	18.98 16.41	17.28	.....	.....	.....
Humic acid, 20 p.p.m. + asparagin	5.1	.....	.....	19.15 20.91	14.93	7.1 6.9
Humic acid, 20 p.p.m. + KNO_3	5.1	.....	.....	12.28 12.50	7.29	7.3 7.2
Humic acid, 100 p.p.m. + asparagin	5.42	20.23 25.10	17.25	.....	.....	.....

* Cultures nearly dead at end of experiment.

Basal medium: First experiment, K_2HPO_4, 0.5 gm.; $MgSO_4 \cdot 7H_2O$, 0.2 gm.; NaCl, 0.2 gm.; $CaSO_4 \cdot 2H_2O$, 0.1 gm.; $FeCl_3 \cdot 6H_2O$, 0.005 gm.; $CaCO_3$, 1.0 gm.; glucose, 10 gm.; H_2O, 1000 cc. pH 7.8. Second experiment, sucrose substituted for glucose.

Light: First experiment, artificial, 18-hour day, 200 foot candles; second, 12-hour day, 300 foot candles.

Incubation period: First experiment, 85 days (11/27/29–2/20/30); second, 78 days (3/21/30–7/7/30).

When media similar to the preceding but without $CaCO_3$ were used, the growth was almost invariably considerably slower, owing in part at least to the lower pH resulting.

UNAERATED CULTURES.—The medium is usually not satisfactory for the growth of the alga in unaerated cultures, but the omission of

TABLE 2

VARIOUS MEDIA FOR USE IN AERATED AND UNAERATED CULTURES

TREATMENT (ADDITIONS PER 100 CC. OF MEDIUM)	UNAERATED				AERATED (1% CO_2 IN AIR)			
	NITROGEN FIXED (MG.)	AVERAGE (MG.)	FINAL pH	FINAL CELL CONDITION	NITROGEN FIXED (MG.)	AVERAGE (MG.)	FINAL pH	FINAL CELL CONDITION
Check (basal medium I only)	1.55		7.3	Normal	5.06		6.6	Normal
	1.43	1.49	7.3	"	5.62	5.34	6.6	"
$CaCO_3$, 20 mg.	2.29		8.0	Poor	7.06		6.7	"
	1.72	2.01	7.8	"	6.97	7.02	6.7	"
$CaCO_3$, 50 mg.	2.32		8.0	"	8.50		6.9	"
	1.87	2.10	8.0	"	5.70	7.10	7.0	"
$CaCO_3$, 100 mg.	2.10		8.0	"	10.18		7.4	"
	1.98	2.04	8.0	"	9.43	9.81	7.6	"
$CaCO_3$, 100 mg. + glucose, 1%	2.14		8.3	Fair	7.58		7.5	"
	1.24	1.69	8.2	"	7.10	7.34	7.3	"
$CaCO_3$, 100 mg. + sucrose, 1%	2.14		8.1	Poor	17.34		7.2	"
	2.14	2.14	8.0	"	19.92	18.63	7.0	"
Glucose, 1% (no $CaCO_3$)	0.06		6.8	Normal	1.32		6.4	Fair
	0.25	0.16	6.8	"	0.73	1.03	6.4	"
Sucrose, 1% (no $CaCO_3$)	2.38		7.2	"	12.40		5.9	Normal
	2.22	2.30	7.3	"	11.19	11.80	6.0	"
Basal medium II only	2.65		6.8	"	2.08		6.6	"
	1.45	2.05	6.8	"	2.38	2.23	6.4	"
Basal medium II + $CaCO_3$, 100 mg.	2.97		8.0	Poor	10.36		7.0	"
	2.55	2.76	8.3	"	10.51	10.44	7.2	"

Basal medium I: K_2HPO_4, 1.0 gm.; $MgSO_4 \cdot 7H_2O$, 0.2 gm.; NaCl, 0.2 gm.; $CaSO_4 \cdot 2H_2O$, 0.2 gm.; synthetic iron humate, 5 p.p.m.; H_2O, 1000 cc. pH 7.4.

Basal medium II: Same as medium I except K_2HPO_4, 0.7 gm. and KH_2PO_4, 0.3 gm. as phosphate buffer. pH 6.9.

Light: Artificial, continuous, approximately 350 foot candles.

Incubation period: 44 days (10/28/33–11/11/33).

the $CaCO_3$ makes it as satisfactory as any yet tested. In the presence of the $CaCO_3$ the initial growth rate is comparatively rapid, but death usually occurs after a few days without aeration unless the

illumination is greatly reduced. Death is due to a rapid increase in pH, as will be shown later. The addition of a suitable sugar usually greatly improves this medium and allows growth in the presence of $CaCO_3$ to continue for a longer period. For instance, table 1 shows a fixation of 9–17 mg. N in 100 cc. of media containing 1 per cent glucose and 0.1 per cent $CaCO_3$ during a period of 85 days. At the

TABLE 3

NITROGEN FIXATION UNDER VARYING LIGHT CONDITIONS AND PARTIAL PRESSURES OF CARBON DIOXIDE

ILLUMINATION (MAZDA LAMPS)		MEDIUM USED	AERATED WITH 1% CO_2 IN AIR			AERATED WITH 5% CO_2 IN AIR		
DAILY PERIOD (HOURS)	APPROXIMATE INTENSITY (FOOT CANDLES)		NITROGEN FIXED (MG.)	AVERAGE (MG.)	FINAL pH	NITROGEN FIXED (MG.)	AVERAGE (MG.)	FINAL pH
24	350	I	18.5	.....	7.2	17.3	.....	7.0
24	"	"	18.0	18.25	7.2	15.2	16.25	6.9
24	175	"	14.5	.....	7.0	11.6	.....	7.1
24	"	"	11.8	13.15	6.9	9.4	10.50	7.0
18	350	"	16.7	.....	7.2	17.9	.....	7.0
18	"	"	13.3	15.00	7.2	17.4	17.65	7.0
24	"	II	6.4	.....	7.2	.....	.....	.....
24	"	"	6.9	6.65	7.3	.....	.....	.....
24	175	"	6.0	.....	7.2	.....	.....	.....
24	"	"	5.0	5.50	7.3	.....	.....	.....
18	350	"	7.2	.....	7.2	.....	.....	.....
18	"	"	6.7	6.95	7.2	.....	.....	.....

Basal medium I: K_2HPO_4, 1.0 gm.; $MgSO_4 \cdot 7H_2O$, 0.2 gm.; NaCl, 0.2 gm.; $CaSO_4 \cdot 2H_2O$, 0.1 gm.; synthetic iron humate, 5 p.p.m.; $CaCO_3$, 10 gm.; H_2O, 1000 cc.

Basal medium II: Same as medium I except K_2HPO_4, 0.2 gm. and $NaHCO_3$, 1.0 gm. added.

Incubation period: 81 days (12/21/33–3/12/34).

end of the experiment the cultures were nearly dead, but it is probable that the sugar was practically all utilized before the cultures began to turn yellow.

ENERGY SOURCES.—Sunlight is a satisfactory source of energy for providing the materials needed for respiration and growth, but usually the rate of growth is accelerated by the addition of suitable sugars. An intensive study of the kinds of sugar used by *Nostoc* has not yet been made, but the two tested, sucrose and glucose, have both increased growth in light. In table 2, the nitrogen fixation in

TABLE 4

GROWTH AND NITROGEN FIXATION AT VARIOUS H ION CONCENTRATIONS

Initial pH	Relative growth (14 days)	Nitrogen fixation (44 days)		Final pH
		Total (mg.)	Average (mg.)	
5.2	None	0 0	0	5.6 5.6
5.5	"	0 0	0	5.8 5.8
5.7	"	0 0.13	0.07	6.0 6.2
6.0	Slight	1.95 1.62	1.79	6.2 6.2
6.3	"	1.65 1.45	1.55	6.3 6.4
6.5	"	1.56 1.23	1.40	6.4 6.4
6.6	"	2.32 1.24	1.78	6.5 6.5
6.8	Fair	1.48 1.51	1.50	6.7 6.7
7.4	"	1.59 1.56	1.58	7.1 7.1
7.6	"	2.34 1.92	2.13	7.4 7.4
8.0	"	1.74 1.89	1.82	7.8 7.8
8.4	"	1.47 2.53	2.00	8.4 8.8

Basal medium: $MgSO_4 \cdot 7H_2O$, 0.2 gm.; NaCl, 0.2 gm.; $CaSO_4 \cdot 2H_2O$, 0.2 gm.; synthetic iron humate, 5 p.p.m.; H_2O, 1000 cc.; potassium phosphates (mono-, di- or tri- to give desired pH), 1.0 gm.

Aeration: Unaerated.

Light: Artificial, continuous, approximately 350 foot candles.

Incubation period: 44 days (10/28/33–11/11/33).

aerated cultures containing no $CaCO_3$ was 5.34 mg. N without sugar and 11.80 with sucrose; the corresponding figures with $CaCO_3$ were 9.81 and 18.63. Glucose in this experiment, as frequently happens, produced a toxic effect, owing to its decomposition in the autoclave when sterilized in an alkaline medium, even though the sterilization lasted for only 10 minutes at 10 pounds' pressure. In an earlier experiment (table 1), however, good growths were obtained in a some-

TABLE 5

PHOSPHATE BUFFERS

PHOSPHATE ADDITIONS TO BASAL MEDIUM (GM. PER LITER)		INITIAL pH	NITROGEN FIXED (MG.)	FINAL pH
KH_2PO_4	K_2HPO_4			
0.1	.....	7.0	8.85	5.8
0.2	.....	6.6	7.91	5.8
0.5	.....	6.4	1.47	5.8
1.0	.....	6.2	0.22	5.8
2.0	.....	6.0	0.26	5.6
5.0	.....	5.4	0.19	5.1
.....	0.1	7.0	6.70	5.8
.....	0.5	7.4	10.45	5.8
.....	5.0	8.2	3.39	7.3
0.05	0.05	7.0	10.15	5.4
0.25	0.25	6.8	7.71	5.6
2.5	2.5	6.8	3.39	6.6
0.09	0.01	7.1	8.52	5.6
0.45	0.05	6.6	4.31	5.8
4.5	0.5	6.0	0.12	5.6

Basal medium: $MgSO_4 \cdot 7H_2O$, 0.2 gm.; NaCl, 0.2 gm.; $CaSO_4 \cdot 2H_2O$, 0.1 gm.; $FeCl_3 \cdot 6H_2O$, 0.005 gm.; $CaCO_3$, 0.2 gm.; H_2O, 1000 cc.

Aeration: Aerated with 1% CO_2 in air.

Light: Artificial, 12-hour day, approximately 300 foot candles.

Incubation period: 66 days (4/22/30–6/27/30).

what similar medium. Separate sterilization of the glucose in a neutral or preferably slightly acid solution is advisable.

NITROGEN SOURCES.—Ammonium nitrate, ammonium sulphate, potassium nitrate, and asparagin have been used in different experiments. The growth with potassium nitrate was usually better than with the other nitrogen sources, probably because it makes the medium more alkaline (table 7). This increase is desirable in cultures aerated with 1 per cent CO_2 in air, of course, but might be very

TABLE 6

EFFECT OF IRON, BORON, AND MANGANESE ON NITROGEN FIXATION

Treatment	First experiment	Second experiment		
	Nitrogen fixed (mg.)	Nitrogen fixed (mg.)	Average (mg.)	Final pH
Check (basal medium without $FeCl_3$)	17.69	6.63 8.73	7.68	7.0 6.9
Check (basal medium)	17.49	8.17 8.48	8.33	7.3 7.3
Ferric citrate, 2 p.p.m. Fe (no $FeCl_3$)	.....	9.03 9.42	9.26	7.2 7.3
Ferric citrate, 20 p.p.m. Fe (no $FeCl_3$)	.....	7.73 7.16	7.45	7.2 7.2
Natural humic acid, 50 p.p.m. (no $FeCl_3$)	.....	7.30 9.32	8.31	7.0 6.8
Natural humic acid, 50 p.p.m.	.....	8.19 10.90	9.55	6.7 7.1
Ferric citrate, 2 p.p.m. Fe (no $FeCl_3$)+ natural humic acid, 50 p.p.m.	.....	8.76 8.58	8.67	7.0 7.0
Boric acid, 0.1 p.p.m.	13.49	.....	.....	.....
Boric acid, 1 p.p.m.	13.79	7.16 7.93	7.55	7.4 7.3
Boric acid, 10 p.p.m.	19.99	.....	.....	.....
Boric acid, 20 p.p.m.	.....	8.62 6.49	7.56	7.1 7.0
$MnSO_4$, 5 p.p.m.	14.79	7.54 6.28	6.91	7.0 7.1
$MnSO_4$, 50 p.p.m.	14.29	9.15 8.34	8.75	6.8 6.8
Boric acid, 0.1 p.p.m.+$MnSO_4$, 5 p.p.m.	11.79	.....	.....	.....

Basal medium: K_2HPO_4, 0.5 gm.; $MgSO_4 \cdot 7H_2O$, 0.2 gm.; NaCl, 0.2 gm.; $CaSO_4 \cdot 2H_2O$, 0.1 gm.; $FeCl_3 \cdot 6H_2O$, 0.005 gm.; $CaCO_3$, 1.0 gm.; H_2O, 1000 cc. (first experiment 200 cc. medium per flask; second experiment 100 cc.).

Aeration: Aerated with 1% CO_2 in air.

Light: Artificial, 12-hour day, approximately 300 foot candles.

Incubation period: First experiment 56 days (4/2/30–5/28/30); second 55 days (6/5/30–7/30/30).

undesirable in unaerated cultures. Ammonium nitrate and ammonium sulphate make the medium sufficiently acid to retard growth or even to kill the culture unless adequate $CaCO_3$ or other buffer is present.

TABLE 7

IMPORTANCE OF CALCIUM AND STRONTIUM IN NITROGEN FIXATION

TREATMENT (ADDITIONS PER 100 CC. MEDIUM)	FIRST EXPERIMENT*		SECOND EXPERIMENT*		THIRD EXPERIMENT*†	
	NITROGEN IN CELLS (MG.)	FINAL PH	TOTAL NITROGEN (MG.)	FINAL PH	TOTAL NITROGEN (MG.)	FINAL PH
Check (basal medium only)	0.87	7.0	1.51	6.7	3.10	6.7
KNO_3, 10 mg. N	.....	.....	.....	.....	8.80‡	7.3
KNO_3, 20 mg. N	5.29	7.8	12.81‡	7.6	.....	.....
$CaSO_4$, 10 mg	.....	.....	2.73	6.7	3.67	6.7
$CaSO_4$, 20 mg	1.32	6.4	.....	.....	.....	.....
$CaSO_4$, 50 mg	.....	.....	1.15	6.2	1.26	6.1
$CaSO_4$, 20 mg.+KNO_3, 20 mg. N	4.51	7.8	.....	.....	.....	.....
$SrSO_4$, 20 mg	1.89	7.0	2.18	6.7	4.32	6.7
$SrSO_4$, 20 mg.+KNO_3, 20 mg. N	4.90	7.9	.....	.....	.....	.....
$CaCO_3$, 14.7 mg	1.88	7.4	.....	.....	.....	.....
$CaCO_3$, 36.8 mg	.....	.....	2.57	7.1	5.43	7.0
$CaCO_3$, 14.7 mg.+KNO_3, 20 mg. N	8.19	8.1	.....	.....	.....	.....
$CaSO_4$, 20 mg.+$CaCO_3$, 14.7 mg	1.45	7.2	.....	.....	.....	.....
$CaSO_4$, 20 mg.+$CaCO_3$, 14.7 mg.+KNO_3, 20 mg. N	6.57	7.9	.....	.....	.....	.....
$CaSO_4$, 20 mg.+$CaCO_3$, 14.7 mg.+asparagin, 20 mg. N	4.19	7.0	.....	.....	.....	.....

* All results are averages of two or more cultures.

† Inoculated with culture grown on calcium-free medium.

‡ Nitrogen in cells only.

Basal medium: K_2HPO_4, 0.75 gm.; $MgSO_4 \cdot 7H_2O$, 0.2 gm.; NaCl, 0.2 gm.; $FeCl_3 \cdot 6H_2O$, 0.005 gm.; H_2O, 1000 cc.

Aeration: Aerated with 1% CO_2 in air.

Light: Artificial, 12-hour day, approximately 300 foot candles.

Incubation period: First experiment 23 days (4/2/30–4/25/30); second 47 days (6/18/30–8/4/30); third 53 days (8/11/30–10/3/30).

ILLUMINATION

The optimum light intensity was found to depend to a marked extent upon the growth conditions, particularly the medium used, amount of CO_2 or sugar available, and possibly upon the degree of

TABLE 8

IMPORTANCE OF CALCIUM IN NITROGEN FIXATION

TREATMENT (ADDITIONS PER 100 CC. MEDIUM)	TOTAL NITROGEN (MG.)	AVERAGE (MG.)	FINAL pH
Check (basal medium only)	0.13 0.10	0.12	6.9 6.9
$CaSO_4$, 20 mg.	0.42 0.45	0.44	6.7 6.7
$MgSO_4$, 20 mg.	0.54 0.13	0.34	6.9 6.9
$CaSO_4$, 20 mg. + $MgSO_4$, 20 mg.	2.95 2.19	2.57	6.6 6.6
KNO_3, 10 mg. N	2.05* 0.46*	1.26*	6.9 6.8
KNO_3, 10 mg. N + $MgSO_4$, 2.5 mg.	4.44* 4.01*	4.23*	7.2 7.2
KNO_3, 10 mg. N + $MgSO_4$, 20 mg.	6.29*	6.29*	7.2
KNO_3, 10 mg. N + $MgSO_4$, 100 mg.	0.87*	0.87*	6.9

* Nitrogen in cells only.

Basal medium: K_2HPO_4, 0.75 gm.; H_2O, 1000 cc.

Aeration: Aerated with 1% CO_2 in air.

Light: Artificial light, 12-hour day, approximately 300 foot candles.

Incubation period: 53 days (8/11/30–10/3/30).

Inoculum: Used culture grown on calcium-free medium consisting of K_2HPO_4, 0.75 gm.; $MgSO_4 \cdot 7H_2O$, 0.2 gm.; H_2O, 1000 cc.

TABLE 9

NITROGEN FIXATION IN THE DARK IN UNAERATED MEDIA

	FIRST EXPERIMENT (98 DAYS)	SECOND EXPERIMENT (188 DAYS)
Number of cultures	15	21
Average nitrogen fixed per 100 cc.	2.6 mg.	2.5 mg.
Average glucose consumed	290.0 "	250.0 "
Average efficiency (N fixed per gm. glucose consumed)	9.1 "	10.2 "
Maximum fixation per 100 cc.	5.4 "	5.6 "
Maximum efficiency	14.8 "	13.2 "

Basal medium: K_2HPO_4, 0.5 gm.; $MgSO_4 \cdot 7H_2O$, 0.2 gm.; NaCl, 0.2 gm.; $CaSO_4 \cdot 2H_2O$, 0.1 gm.; $FeCl_3 \cdot 6H_2O$, 0.005 gm.; glucose, 10 gm.; H_2O, 1000 cc. Additional buffers ($CaCO_3$, K_2HPO_4, or K_3PO_4) supplied in a few flasks for adequate control of pH.

aeration. Under most and possibly all conditions direct sunlight seems to be too intense an illumination for best growth, although good growths and nitrogen fixation have been obtained when cultures were exposed to direct sunlight in the laboratory window for four–six hours daily. A considerably lower light intensity is certainly preferable. The diffuse light of an ordinary well lighted room is very satisfactory for the maintenance of cultures in a healthy condition. The writers have kept a few liquid cultures in cotton-stoppered flasks under such conditions for about six years without transfer to fresh media, and they are still in remarkably good condition.

The light intensities chosen for use in these investigations, after several preliminary experiments, ranged from 175 to 350 foot candles, usually near 300 foot candles. Even this intensity sometimes seemed too high, and hence the experiment reported in table 3 was carried out. It will be observed that in the phosphate medium continuous light of an intensity of 175 foot candles in comparison with 350 foot candles produced on the average about 68 per cent as large a nitrogen fixation. Observations during growth, however, showed that the initial growth (where 1 per cent CO_2 was used) was much more rapid at the higher light intensity. These cultures showed a tendency to turn slightly yellow after a few weeks, while the cultures in the lower light remained green. With 5 per cent CO_2 the growth rate was slower, especially at first, as will be explained in a later section.

There were certain indications throughout these investigations that the alga needs a daily rest period of non-illumination, and that a 12- to 18-hour daily period of illumination is preferable to continuous light. The data of table 3 report a direct experiment to study this point. It may be seen that there were no very marked differences in the final results between an 18- and a 24-hour day. The 18-hour cultures seemed to be slightly better than the others maintained at the same light intensity, but not better than those under continuous light at the lower light intensity. Evidently these organisms do not require a rest period if the light intensity is sufficiently low and the growth conditions are satisfactory. The favorable effect of the rest period, sometimes observed, is probably due in large measure to the decrease in total daily illumination. In work with *Chlorella* a 12-hour

day is commonly used, and probably a similar daily period of illumination for *Nostoc* might prove most satisfactory for most experiments, especially when continued for several weeks.

Since the light from a Mazda lamp contains a much higher percentage of long rays than does sunlight, tests were made to determine whether the elimination of these would affect growth favorably. When glass filters which removed the infra red and a considerable portion of the visible red rays were used, a somewhat slower growth rate resulted, due in all probability to the reduction in total illumination. The filters were therefore not used in later experiments.

Carbon dioxide supply

Unaerated cultures, containing no sugar in the medium, make a relatively poor growth, especially if air is the only source of CO_2. This slower growth of unaerated cultures, assuming that the pH remains satisfactory, is due chiefly to lack of CO_2, since the addition of glucose to such unaerated cultures will permit good growth. Qualitative observations indicate that the oxygen supply may also be inadequate for maximum growth in unaerated cultures, but further experimentation would be necessary to establish this point. In most of the experiments a cylinder of compressed air containing 1 per cent CO_2 was used for aeration. In table 3 are reported comparative nitrogen fixations using 1 and 5 per cent CO_2. Generally speaking, 1 per cent proved more satisfactory, chiefly because of the more favorable pH of the medium. Under these experimental conditions the initial pH of the cultures receiving 5 per cent CO_2 was usually about 0.3 to 0.5 pH lower than when 1 per cent CO_2 was used.

Observations during the growth of the cultures (table 3) showed that during the first three or four weeks most of the cultures aerated with 5 per cent CO_2 made very little growth, whereas with the lower partial pressures of CO_2 the growth was excellent. During the second month there was a marked improvement in the cultures receiving 5 per cent CO_2, and at the time of analysis they were about as good as those receiving 1 per cent CO_2. Since the difficulty in keeping a culture at pH 7 or above increases with CO_2 concentration, there would seem to be no adequate reason for using air containing more than 1 per cent CO_2.

HYDROGEN ION CONCENTRATION

LIMITS OF GROWTH.—A neutral or alkaline medium is decidedly preferable for this species of *Nostoc*. Table 4 shows that growth does not take place below a pH of approximately 5.7. This figure has been checked in aerated and unaerated cultures and invariably the lower limit of growth initiation has been between 5.7 and 5.9. Likewise, when growing cultures show signs of death because of increasing H ion concentration, the pH observed at the first signs of yellowing is usually between 5.5 and 5.7, slightly lower than the limit of growth initiation, as would be expected. No study has yet been made to determine whether *Nostoc* can grow at a considerably lower pH if given combined nitrogen, as BURK (10) observed in the case of *Azotobacter*.

Table 4 also shows that normal growth occurs at a pH of 8.4 to 8.8. The exact upper limit for growth has not yet been determined but apparently it is at a pH of approximately 9. As will be shown later in figure 9, if the alga is grown in unaerated media in the presence of carbonates the pH may rise to 9.4–9.6, killing practically all vegetative cells.

OPTIMUM PH.—Growth is usually best at the pH range of about 7.0 to 8.5, and decreases in going from pH 7 to 6, especially toward the lower limit. This is not shown by the results in table 4 because these were unaerated cultures, but is based upon the general observations made during the course of these studies. When the pH was such as to allow growth to be initiated, then at the end of 44 days the fixations were nearly the same in all flasks, but the nitrogen fixed was small (table 4). If an adequate supply of CO_2 had been available, such uniform fixations would not have been obtained.

BUFFER SALTS.—The buffer used in most of the experiments was K_2HPO_4. In a synthetic medium such as recommended on a previous page, but containing no carbonate, the K_2HPO_4 gives a pH of about 7.2–7.4, which is optimum. Aeration with 1 per cent CO_2 reduces the pH to about 7.0. If 0.1 to 1.0 per cent $CaCO_3$ is present, however, the pH is usually maintained at about 7.2 to 7.5 when so aerated.

Table 5 gives the results of a study of different proportions and quantities of mono- and di-potassium phosphate buffers. Too much

emphasis should not be placed upon the final pH values, since older cultures, containing dead cells, frequently show a low pH. The greatest nitrogen fixation was obtained with 0.05 per cent K_2HPO_4, while a concentration of 0.5 per cent was somewhat toxic. In other experiments there were some indications that even a concentration of 0.1 per cent may be above the optimum.

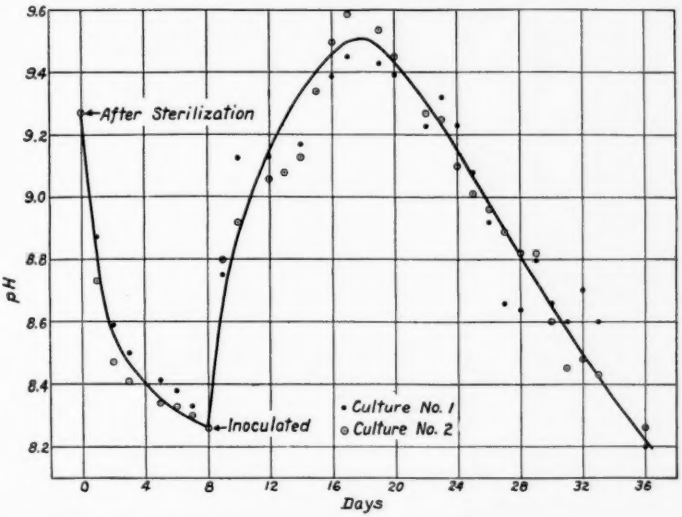


FIG. 9.—Effect on pH caused by addition of heavy inoculum of *Nostoc muscorum* to unaerated inorganic medium containing 1 per cent $CaCO_3$ and exposed to continuous light.

Other buffers, such as $NaHCO_3$ (table 3) and Na_2HPO_4, were used in a few experiments but with less satisfactory results, at least at the concentrations chosen. $NaHCO_3$ cannot be used satisfactorily in unaerated culture media because of the marked rise in pH, similar to that which occurs with $CaCO_3$.

CARBONATES AND PH.—Figure 9 gives results showing the rise in pH brought about by algal cultures growing in an inorganic medium containing 1 per cent $CaCO_3$ and unaerated. The H ion determinations were made by means of the glass electrode, using Ag-AgCl against HgCl-Hg with saturated KCl as the conducting bridge. The

culture vessels (500 cc. balloon flasks) contained 100 cc. portions of the sterile medium with the sterile glass electrodes immersed in it. Continuous light of about 400 foot candles intensity was used and the temperature maintained at 27°–31°C. After eight days, when the pH of the medium had become fairly constant, heavy inocula of the algal mass were added; otherwise the flasks were left undisturbed throughout the experiment. The daily readings of duplicate cultures are given in figure 9, with the curve drawn so as to represent the average result.

The freshly autoclaved medium had a pH of 9.27, owing undoubtedly to the driving off of CO_2 through heating. After standing undisturbed for eight days in contact with air the pH had dropped to 8.26. As soon as the heavy inocula of the alga were added the pH began to rise rapidly, reaching a maximum of 9.45–9.59 on the ninth day subsequent to inoculation. This killed all or practically all of the actively growing cells and hence the pH then began a gradual decline. Nineteen days later it was back at approximately the same point as when inoculated. In other similar experiments, where small inocula were used, the rise in pH to the death point under continuous illumination required about three weeks.

Figure 9 shows that it is the removal of CO_2 from $CaCO_3$ by the actively photosynthesizing culture, with the resulting formation of the hydroxides of calcium and the other basic elements present, that results in the rise in pH. It is perhaps surprising that the alga will continue to photosynthesize up to the point where the resulting high alkalinity causes death. The pH of algal cultures containing dead cells was somewhat slower in returning to the original value, 8.2, than that of the freshly sterilized medium. This was probably due primarily to the fact that a few algal cells were not killed and were able to grow slowly when the alkalinity had decreased sufficiently. The very slow recovery of cultures which earlier appeared to be dead from high pH has been observed frequently.

Temperature

The temperature of 24°–27° C. used in most of the experimental work reported here was satisfactory. The optimum has not been determined accurately, but over the range of 25°–30° C. no great difference

in the rate of growth was observed. At higher temperatures, especially at 35°–40° C., the growth rate was much slower and the cells, when observed under the microscope, frequently appeared to be abnormal. At the lower temperatures (5°–10° C.) the vegetative cells commonly go into a resting condition, in which they will remain dormant for several months. When taken out of the refrigerator active cell division begins within about a week. These resting filaments are not true spores, but resemble ordinary vegetative filaments except that the cells become somewhat larger and distinctly egg-shaped.

Effect of soil humic acid

Natural humic acid frequently acts as a growth stimulant for a variety of plant types, including both higher green plants and microorganisms. Recent work (**2, 10, 11, 12, 21**), carried out in this laboratory, has shown that the cause of this stimulation may vary for different types of plants, and even for the same plant grown in different media. In the case of higher plants the stimulation is usually due chiefly to the available iron added. This humic acid iron does not precipitate readily, even in an alkaline medium, which is in distinct contrast to the behavior of inorganic iron salts. Certain trace elements, present in the humic acid, may also often be responsible in part for the increased growth of higher plants. In the case of *Azotobacter* the humic acid effect is usually due to the addition of available iron, molybdenum, or vanadium. Certain strains of rhizobia frequently show an exceptionally large growth increase following additions of natural humic acid. This was shown (**2**) to be due chiefly to the presence of "coenzyme R," an essential growth substance required by these bacteria.

The results of studies to determine the response of *Nostoc* to natural humic acid are given in tables 1 and 6. The humic acid was prepared by the extraction of a fertile soil with alkali, followed by precipitation with acid and re-solution with alkali, according to the usual methods.

Nitrogen fixation was increased by the highest rate of application (table 1) from 9.07 to 17.28 mg. N per 100 cc. This large increase occurred in an unaerated medium containing $CaCO_3$ and having an

initial pH of 7.8. The results of the second experiment (table 1) agree with those of the first, even though these cultures were aerated with 1 per cent CO_2 and the pH was only slightly alkaline. In table 6, however, the effect of humic acid, as well as of ferric chloride and ferric citrate, was small. In the two experiments where humic acid gave the greatest effect the media contained sugar, while in the other case no sugar was present. The incubation periods were longer and the total growth likewise larger in the presence of the sugar. No attempt has yet been made to determine the primary cause of the humic acid stimulation, but it seems probable that the available iron is of greatest importance.

Tests with boron and manganese

In table 6 are given the results of tests of boron and manganese on growth and nitrogen fixation. There was no indication that either of these elements is needed in quantities greater than those found as impurities in the medium.

Calcium and strontium in nitrogen fixation

It was recently reported from this laboratory (13) that either calcium or strontium is essential for nitrogen fixation by *Azo. vinelandii*, but not for growth in the presence of an adequate supply of fixed nitrogen. Similar tests with *Nostoc* are reported in table 7. No attempt was made to free the basal medium of the last traces of calcium and strontium.

The results of the first experiment reported in table 7 would seem to indicate that neither calcium nor strontium, at least in concentrations greater than the traces present as impurities, is needed for growth. Some nitrogen fixation also occurred in the absence of added calcium or strontium, but at a considerably reduced rate. The effects of $CaSO_4$, $CaCO_3$, and $SrSO_4$ on nitrogen fixation were essentially the same, but $CaCO_3$ produced an appreciably better growth in the presence of fixed nitrogen than did the other compounds. The results of the second and third experiments (table 7) agree essentially with those of the first.

In further studies, reported in table 8, a basal medium consisting of only K_2HPO_4 was used and the inoculating culture was again

grown on a calcium-free medium. These data show that magnesium is essential for both growth and nitrogen fixation, as would be expected since this element is a constituent of chlorophyll. In the medium containing K_2HPO_4 and $MgSO_4$ practically no growth was obtained, except where either combined nitrogen or $CaSO_4$ was added. The small growth obtained under these conditions, corresponding to 0.22 mg. N, can be accounted for on the basis of the calcium impurity in the $MgSO_4$. The addition of $CaSO_4$ to this medium brought about normal growth and a fixation of nitrogen amounting to 2.57 mg. N, or approximately half as good a growth as with KNO_3 and no calcium. Although no flasks were included in this particular experiment with both $CaSO_4$ and KNO_3 present, it may be stated fairly definitely from other experiments that the growth under these conditions would not have been more than 50 per cent greater than with the nitrate in the absence of calcium.

These data suggest that calcium is essential for nitrogen fixation but probably not for growth. In the case of the results reported in table 7, it is probable that the traces of calcium present in the medium were adequate for the fixation obtained.

In another similar experiment, except that $NaHCO_3$ was used as a buffer instead of K_2HPO_4, the necessity for a supply of phosphorus for growth was shown conclusively.

Nitrogen fixation in the dark

Nostoc muscorum will live and grow for months in the dark and form normal appearing chlorophyll if given a suitable energy source, such as glucose. Growth and nitrogen fixation take place rather slowly but the quantities fixed per unit of energy consumed are usually large. Table 9 gives a summary of the results of two experiments carried out in the dark except for brief growth observations at intervals of about one month. In these experiments a basal inorganic salts medium containing 1 per cent glucose was used. $CaCO_3$ was added to a few flasks and additional phosphate was supplied to others in order to increase the buffering capacities. Several of the cultures not supplied with $CaCO_3$ were nearly dead when analyzed, owing to the increase in acidity, the pH in these cases usually being 5.4–5.8. This increase was undoubtedly due to organic acids formed,

since growth in the presence of $CaCO_3$ continued normally throughout the incubation period. In these flasks the final pH was near 7 and the largest fixations and usually the highest efficiencies were obtained. An average fixation of 9.6 mg. N per gram of glucose consumed, with a maximum value of 14.8 mg. N under ordinary atmospheric conditions of growth, is higher than commonly obtained with *Azotobacter* or other nitrogen-fixing bacteria.

Growth cycle as factor in physiological studies

These studies, considered as a whole, emphasize the difficulty sometimes experienced in securing close agreements between experiments carried out from time to time under similar but not necessarily exactly identical experimental conditions. Considerable time was spent in determining the causes for these growth variations. Aside from the numerous points already considered, such as pH, CO_2 concentration, and light intensity, the growth cycle of the organism should not be overlooked. In the case of bacteria, where growth consists largely of cell enlargement followed by cell division, the process is relatively simple. In *Nostoc* the growth cycle may vary from time to time. Perhaps the most common cycle is for resting vegetative cells, upon renewing their activity, to divide, thus increasing the length of the trichomes. After a growth period of two to five days these cells commonly break at the heterocysts or separation discs, forming young hormogones which are actively motile for a few hours, during which time they become long and slender. They may then come to rest, the cells increase about twofold in diameter, and heterocyst formation occur. Cell division of these trichomes may or may not continue uninterrupted. These trichomes may again form new hormogones, they may come to rest and remain so for several days, or they may break up into jelly-like masses consisting of the individual cells held together by the gel and showing little resemblance to the original filamentous arrangement. These single cells may in turn divide and become new hormogones. Under other conditions the organism may go into the spore stage, frequently practically all vegetative cells showing such a change. These spores may, of course, develop into new trichomes. As a result of these and other possibilities, cultures when examined under the microscope

may show marked differences in appearance from day to day, and it is not surprising that growth rates may vary markedly. The appearance of the organism in older cultures in contrast to its appearance when young is especially marked. This suggests that most quantitative work done with this organism with a view to establishing functional relations might profitably be limited to much shorter incubation periods than used in most of the experiments reported here.

Importance of blue-green algae in soil

Practically all cultivated soils and grasslands have a rather abundant flora of blue-green algae, various species of *Nostoc* being especially common. In fact the majority of the members of this genus are terrestrial. Bristol (7), Moore and Carter (28), and Lowe and Moyse (23) reported the finding of *Nostoc muscorum* in soils. Additional studies dealing with the occurrence of blue-green algae in soils were reported by Esmarch (16, 17), Robbins (31), Bristol (8, 9), and Petersen (29). Russell (32) emphasizes the importance of algae, especially blue-greens, in the colonization of new ground following volcanic eruptions. Petersen (29) points out that the limited data available at present indicate that a gram of soil usually contains about 10,000 to 100,000 algae (greens and blue-greens). Calculations of the actual mass of algal cells in soil show that their weight is often almost as great as that of the bacteria.

Considerable work has been reported in which attempts were made to determine whether algae can fix free nitrogen. Moore and Carter (28), Drewes (15), Jones (22), and Petersen (29) have reviewed the literature. It has been shown repeatedly that substrates frequently show gains in nitrogen where algal growths are abundant. Some of the earlier workers, such as Beijerinck (5) in 1901, were convinced that blue-green algae can utilize free nitrogen gas, but they did not work with bacteria-free cultures. Likewise Moore and Webster (26), and Moore, Whitley, and Webster (27) held similar views but did not prove them. Attempts at proof, using pure cultures, including a *Nostoc* sp., were made by Pringsheim (30), Glade (20), and Maertens (24) with negative results. The view then became rather prevalent that none of the algae can utilize nitrogen gas. Even as late as 1930, Jones (22) concluded

from work with three species of the Cyanophyceae that the algae live in association with nitrogen-fixing bacteria to the mutual benefit of both organisms. Work in this laboratory (4) with eight cultures of the Cyanophyceae, obtained from PRINGSHEIM, showed no nitrogen fixation. The work of MOLISCH (25), VOUK and WELLISCH (34), DREWES (15), and COPELAND (14), previously mentioned, as well as the results obtained at this laboratory (1, 3, 4) with *Nostoc muscorum*, stand out in contrast to these negative results. Evidently the ability to fix nitrogen is not a universal characteristic of all the Cyanophyceae but is probably limited to a few genera and species.

One of the most striking points brought out by the researches reported here, as well as in earlier publications from this laboratory, is the comparatively large quantities of nitrogen fixed by *Nostoc muscorum* in a given length of time. Fixations of 10 to 15 mg. N per 100 cc. of inorganic culture medium, aerated with air containing CO_2, were frequently obtained during an incubation period of two to three months, starting with a small inoculum. This is a ten- to twenty-fold greater fixation than reported for other blue-green species by the investigators just mentioned. In old cultures, at least, a considerable portion of this nitrogen was found (4) to be present in a soluble form. In the dark, nitrogen fixation proceeds slowly but the food supply is utilized comparatively efficiently.

A summation of our knowledge of the occurrence and role of blue-green algae in nature would seem to indicate that these organisms are of considerable importance in the maintenance of soil fertility. A similar conclusion was reached by BRISTOL (8), even though the nitrogen-fixing ability of certain Cyanophyceae had not been demonstrated conclusively at that time. PETERSEN (29) is doubtful as to the economic importance of algae in soil, basing his views largely on the supposition that they make little growth except at the soil surface. He considers that the Cyanophyceae, with the exception of *Nostoc punctiforme* and possibly a few others, cannot grow heterotrophically or fix nitrogen in the dark. Our results with the most active nitrogen-fixing blue-green alga yet isolated from soil, together with the recent results of WINTER (35) with *Nostoc punctiforme*, definitely contradict these two ideas which serve in large part as a basis for PETERSEN'S viewpoint. Whether *Nostoc muscorum* actually

makes an appreciable growth in soil where light does not penetrate remains to be determined, but it is at least of interest to know that it has the ability to do so.

If the results reported here with the one species are typical, it would seem that the nitrogen-fixing blue-greens thrive best in nearly neutral or alkaline soils, preferably partly shaded, and where moisture is abundant. *Nostoc* also grows abundantly in fresh water. These studies suggest that even in acid soils it may be able to continue to multiply at the surface, because by growing in colonies and constantly removing CO_2 from the soil during photosynthesis it may increase the pH locally. Its gelatinous covering also enables the organism to withstand remarkably dry soil conditions, as FRITSCH (**18**) and others have pointed out.

The nitrogen-fixing algae, growing near the soil surface, are unique in being able to obtain both their carbon and nitrogen from the air. This, of course, explains why they appear so soon on new volcanic soils and in other places where the soil is too poor to support most other forms of plant life. The quantity of organic matter added as a result of such growth is small but may be of considerable importance in the improvement of such soils.

The algae, like other organisms, compete with higher plants for mineral foods. This competition, while possibly harmful at times, is more likely to have an over-all beneficial effect through the prevention of leaching and the keeping of a reserve supply of mineral elements in a semi-available form for the use of higher plants.

We know comparatively little about the various other chemical transformations which the Cyanophyceae may bring about, but the evidence indicates strongly that per gram of weight they are far less active than common soil bacteria and fungi.

Our information concerning the nitrogen-fixing endophytic blue-green algae is still too limited to permit judgment as to their value in adding to nature's supply of combined nitrogen.

Summary

1. These pure culture studies show the conditions most favorable for growth and nitrogen fixation by the common blue-green alga, *Nostoc muscorum* Ag., previously isolated from soil, freed from other

organisms, and its nitrogen-fixing ability demonstrated. A detailed morphological description of the organism is given.

2. The organism readily obtains both its nitrogen and carbon from the air. Its rate of growth and nitrogen fixation is ten- to twenty-fold greater than that previously reported for other nitrogen-fixing blue-green algae. The quantity of nitrogen fixed is as high as 10 mg. N in 45 days and 18 mg. N in 85 days per 100 cc. of a medium containing no carbohydrate.

3. When kept in the dark in a medium containing glucose, normal appearing chlorophyll forms and growth and nitrogen fixation proceed slowly. A fixation of 10 to 12 mg. N per gram of glucose consumed is common under these conditions.

4. The organism thrives in a variety of media, provided the pH is satisfactory. The lower and upper limits of growth are approximately 5.7 and 9.0, respectively, while the optimum covers the range of about 7.0 to 8.5.

5. A medium containing the usual inorganic salts, without carbonates and sugar, and with K_2HPO_4 as a buffer, is satisfactory for use in unaerated cultures. Growth and nitrogen fixation are much more rapid if the medium is aerated, preferably with air containing about 1 per cent CO_2. The addition of sugars increases the rate of growth of both aerated and unaerated cultures, especially the latter; likewise combined nitrogen, such as nitrates, ammonia, and asparagin, is usually used somewhat more rapidly than is free nitrogen gas.

6. In unaerated liquid cultures containing an abundance of carbonates and exposed to a high light intensity, the pH rises to about 9.6 and most of the cells die. This results from the utilization of the carbonates as a source of CO_2 when neither adequate CO_2 gas nor sugar is supplied.

7. A light intensity of 175 to 350 foot candles from Mazda lamps gives satisfactory results. A 12-hour day is somewhat preferable to continuous illumination except at the lower light intensities.

8. Neither calcium nor strontium, at least in concentrations greater than traces, is necessary for growth in the presence of combined nitrogen. In a nitrogen-free medium nitrogen fixation markedly decreases in their absence, however, suggesting that they play a cata-

lytic role in nitrogen fixation, as in the case of *Azotobacter*. Natural humic acid increases growth and nitrogen fixation, probably due chiefly to its content of available iron. Boron and manganese have no appreciable beneficial effect.

9. These studies indicate that *Nostoc muscorum* is an organism of considerable importance in soils and possibly in fresh ponds and lakes, adding both nitrogen and organic matter to its growth medium. A brief general discussion of the economic importance of the Myxophyceae in soils is given.

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BUREAU OF CHEMISTRY AND SOILS
U.S. DEPARTMENT OF AGRICULTURE
WASHINGTON, D.C.

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NUTRITIONAL LEVELS IN THE PEANUT PLANT

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 477

RUFUS H. MOORE

(WITH TWO FIGURES)

Introduction

PETTIT (12) has discussed the morphology, growth responses, and reproduction of the peanut plant. WALDRON (23) added information concerning the general morphology and physiological response. In both papers the plant is considered indigenous to Brazil, and other species of the Leguminosae which form at least part of their fruits below the surface of the soil are described. REED (17) emphasized the developmental morphology of the fibrovascular bundle, gametophyte, and embryo. Recently STOKES and HULL (19) and HUSTED (4) have reported upon the genetics and cytology of cultivated varieties.

Investigation

The present investigation concerns the responses of the peanut plant to the variations in the supply of light and available nitrogen it may receive. More specifically the planes of nutrition were varied in order to produce definite type responses as follows: (1) complete suppression of reproductive activity, induced by an abundant supply of readily available nitrogen and a very limited accumulation of carbohydrates; (2) production of flowers and gynophores, but not of fruits, induced by an abundant supply of less readily available nitrogen and a slightly increased accumulation of carbohydrates; (3) an abundance of flowers, gynophores, and fruits, induced by a reduction in the supply of available nitrogen and an increased accumulation of carbohydrates; (4) production of flowers and gynophores but not of fruits, induced by a much restricted supply of available nitrogen and a further accumulation of carbohydrates; and (5) complete suppression of reproductive response, induced by still greater restriction of available nitrogen and a still greater accumulation of carbohydrates.

Essentially these five type responses were secured in the cultural treatments summarized in table 1.

Seed of the Spanish variety, strain 18-38-M-9, selected for thirteen successive generations by the United States Department of Agriculture, was secured. To insure freedom from disease organisms and nodule-forming bacteria, bright and unbroken pods were treated with a 0.25 per cent solution of Uspulun for three successive half-hour periods. As soon as the pods were sensibly dry the seeds were shelled. Seeds were used only from pods which had remained dry inside during the treatment.

TABLE 1

TYPE RESPONSES IN RELATION TO CULTURAL TREATMENTS

Type response	Cultural treatments			
	Series	Source of nitrogen	Length of day	Number of plants
1	A	NH_4	6 hours	48
2	AA	NH_4	Seasonal	12
2	B	NO_3	8 hours	48
3	C	NO_3 and NH_4	Seasonal	48
4	D	NO_3	Seasonal	48
5	E	None	Seasonal	48

NUTRIENT SOLUTIONS.—Certain modifications of the more or less standard nutrient solutions were necessary or useful. Seedling peanut plants were found to require a minimum concentration of calcium salts of 0.0006 gm. mols per liter.[1] Healthier and more vigorous seedlings were produced by using ammonium nitrogen than nitrate nitrogen (9). Concentrations of the phosphate ion ordinarily used in nutrient solutions were found to be injurious to the plant when grown in the light; those grown in darkness are not subject to phosphate toxicity. It was found possible to apply the ammonium nitrogen nutrient solution from a single siphon in drip-culture by reducing the concentrations of the calcium and phosphate ions.

GENERAL REPRODUCTIVE BEHAVIOR.—Under the conditions of light and temperature prevailing in Chicago during the spring, the

[1] Reference to the concentrations of salts in nutrient solutions will always be made on this basis.

peanut plant begins to bloom from four to six weeks after planting. The pre-blooming stage can be extended a week or more by the use of ammonium nitrogen.

From one to eight sessile flowers form in the axil of each leaf. These usually open early in the morning, pollination occurs, and the petals generally begin to wither before the day is over. Thus it is possible to make accurate counts of the number of flowers produced. After several days, the fruit stalk or gynophore begins to elongate noticeably, the dried remains of the floral envelope and included organs often capping the ovary. Elongation of the gynophore is initiated by cells at the base of the ovary (**12**). The meristem formed here persists until the ovary is pushed a few centimeters below the surface of the soil, or in case the substratum is not reached, until the gynophore is 10 to 15 cm. in length. During the growth of the gynophore the ovary does not increase perceptibly in size. Usually it does not begin to enlarge until it has been buried in the substratum.

The period between the opening of a flower and the beginning of elongation of its gynophore will be referred to as the flower-to-gynophore interval. Data for plants grown in 1933 are summarized in table 2.

RESPONSES TO PHOTOPERIOD AND AVAILABLE NITROGEN.—The peanut, like the tomato, was found to be an indeterminate bloomer. A jar of six plants blossomed abundantly when continuously illuminated for several weeks. Other plants subjected to three- or four-hour periods of daylight under a muslin canopy blossomed sparingly, until approaching exhaustion of their carbohydrates induced progressive death of leaves.

Responses to available nitrogen were studied in 1933 and 1935. In 1933 four series of plants were grown without shading (table 2). Although the concentration of $Ca(NO_3)_2$ supplied to the several series varied over a wide range, all plants were fruitful. Vegetative extension and the size of the crop of fruit were directly correlated with the available nitrogen given. Chemical analyses were made on ovendried tissues (**6, 7**) of only the highest and lowest nitrogen series. Chemical data are of some interest (table 3), as the plants were dissected into a greater number of fractions than they were for the analyses of 1935 (tables 6–9).

Culture of plants for main problem

To grow plants representing the five type responses chosen, the following experiments were conducted in 1935.

All plants received the same treatment from the time of seeding until the specific cultural treatments were begun. On April 12, treated seeds were sown thickly in white quartz sand which had been put into glazed 3-gallon jars, washed with an acid solution, flushed

TABLE 2

Flower-to-gynophore interval (1933)

Series	Concentration of $Ca(NO_3)_2$	Flowers producing gynophores		Flower-to-gynophore interval in days	
	Grams mols per liter	Number*	Anthesis	Average	Mode
High nitrogen	0.01500	108	July 16–31	12.7	7
		156	Aug. 1–23	8.8	7
Medium nitrogen	0.00450	190	July 16–31	17.0	9
		314	Aug. 1–23	11.0	9
Low nitrogen	0.00225	64	July 16–31	16.4	9
		148	Aug. 1–23	10.9	9
Very low nitrogen	0.00113	61	July 16–31	11.3	9
	0.00032	None	Aug. 1–23	.....	.....

* Totals for four plants from each series.

with distilled water, sterilized in an autoclave, and held at 28°C. during germination. Four days later, when the cotyledons were just emerging, six seedlings as nearly uniform as possible in appearance were transplanted to each jar containing sand prepared as indicated for experimentation. To stimulate vegetative growth, nutrient solution I (table 4) was applied daily for two weeks. On April 26, solution II was substituted and the concentration of its salts was doubled at the end of a week. Manganese, as $MnSO_4$, was applied at about 0.4 p.p.m. and boron, as H_3BO_3, was applied at about 0.3 p.p.m., each time nutrient solutions were given. Iron, as $FeCl_3$, was applied once or twice weekly at the rate of 1–2 p.p.m. for series showing

little vegetative growth and at 5 p.p.m. for those showing extensive growth.

Because of the early onset of blossoming, specific cultural treatments were established when the plants were a little less than four weeks old. At that time the plants were healthy and vigorously

TABLE 3

SUMMARY OF CHEMICAL ANALYSES OF HIGH AND VERY LOW NITROGEN SERIES (1933)*

Series	Fibrous roots	Primary roots and hypocotyls	Stems	Stem tips	Petioles	Leaflets	Flower-bud composite	Gynophores		Fruits		
								Non-fruiting	Fruit bearing	Small	Large	
											Shells	Seeds
	Total sugars											
High N	2.16	4.09	5.13	1.53	1.53	1.12	2.34	1.65	3.79	11.16	10.73	2.87
Very low N	8.76	7.62	4.95	2.17	1.25	1.59	2.27	1.83	3.47	3.92	11.66	5.04
	Starch and dextrins											
High N	0.37	7.01	7.77	2.81	1.64	6.01	5.03	9.00	17.60	10.03	2.94	8.52
Very low N	1.99	11.86	7.89	7.97	10.66	26.23	6.16	5.98	8.14	17.06	2.83	15.32
	Total sugars, starch, and dextrins											
High N	2.53	11.10	12.90	4.34	3.17	7.13	7.37	10.65	21.39	21.19	13.67	11.39
Very low N	10.75	19.48	12.84	10.14	11.91	27.82	8.43	7.81	11.61	25.98	14.49	20.36
	Ether extract											
High N	.....	.....	.....	.....	.....	.....	.....	1.33	0.73	1.40	0.59	44.96
Very low N	.....	.....	.....	.....	.....	.....	.....	1.45	0.85	2.45	trace	47.02
	Total nitrogen plus nitrates											
High N	1.82	1.20	1.07	4.51	1.57	3.38	2.70	3.11	1.97	5.36	1.89	4.45
Very low N	1.52	0.70	0.64	1.47	1.01	1.74	1.70	2.30	1.39	3.96	0.93	2.90

* All figures are for percentages of dry weight. Analytical methods were the same as for 1935 except that total nitrogen plus nitrates were determined by the salicylic acid method.

vegetative, each having seven to nine fully expanded leaves. To avoid the complication of fruit setting before the plants in series A, B, D, and E had reached the desired level of nutrition, flower buds or freshly opened flowers were removed for a time. Daily counts were taken of all flower buds or flowers produced whether or not these

were removed or allowed to remain on the plants. Gynophores were counted periodically in the preceding four series.

SERIES A: VERY HIGH NITROGEN, NON-FRUITFUL.—Each jar of this series was given nutrient solution A, continuously supplied (21) from one siphon. The solution was collected in 18-liter carboys as it drained from the jars, the pH of 7.1 restored with dilute NH_4OH, and the solution used again. Every seven to nine days the jars were thoroughly flushed with tap water, fresh nutrient solution allowed

TABLE 4

NUTRIENT SOLUTIONS

NUTRIENT SOLUTION	CONCENTRATION OF SALTS (GM. MOLS PER LITER)							pH AT APPLICATION
	$(NH_4)_2SO_4$	NH_4NO_3	$CaCl_2$	$Ca(NO_3)_2$	KH_2PO_4	KCL	$MgSO_4$	
I	0.00010	.....	0.00090	.....	0.000225	0.000225	0.00045	6.9
II	0.00040	.....	0.00040	0.00040	0.000045	0.00060	0.00060	.....
A	0.0060	.....	0.00225	.....	0.001125	0.0030	0.0030	7.1
AA	0.0090	.....	0.00225	.....	0.001125	0.0030	0.0030	7.3
B	.....	.....	.....	0.0090	0.001125	0.0030	0.0045	4.5
C-I	.....	.....	.....	0.0045	0.001125	0.0030	0.0045	4.0
C-II	.....	0.00079	0.00079	0.00315	0.001125	0.0030	0.0030	5.3
DE	.....	.....	0.00180	.....	0.000090	0.00180	0.00180	4.3
High N, 1933	.....	.....	.....	0.01500	0.002250	0.002250	0.00450	4.1
Very low N, 1933*	.....	.....	.....	0.00032	0.000080	0.000080	0.00016	4.1

* Formula for the nutrient solution used beginning August 1.

to drain through the sand, and the supply carboys filled with fresh nutrient solution for the next period. During the seven- to nine-day periods of repeated use of the nutrient solution, about 3 per cent of the ammonium nitrogen was daily oxidized to nitrate nitrogen.

The plants were given a six-hour day, from 8:30 A.M. to 2:30 P.M. Light intensity was reduced by muslin stretched 4 feet above the plants. During heavily clouded weather the canopy was rolled up. On very bright days it was supplemented with one or two layers of longcloth.

SERIES AA: VERY HIGH NITROGEN, LIMITED FRUITFULNESS.—Only two jars of plants were included in this test. Nutrient solution AA was supplied by the continuous application method used for

series A. Originally the former series was intended as a guide to determine whether the concentration of 0.0060 for $(NH_4)_2SO_4$ planned for series A would be toxic. As the plants remained healthy the test was continued.

The plants were shaded by muslin about 1 foot above the rim of the jars and were exposed to the seasonal length of day. Muslin protected the plants from direct sunlight during the early morning and late afternoon.

SERIES B: HIGH NITROGEN, LIMITED FRUITFULNESS.—This series received nutrient solution B. The plants were moved out of the

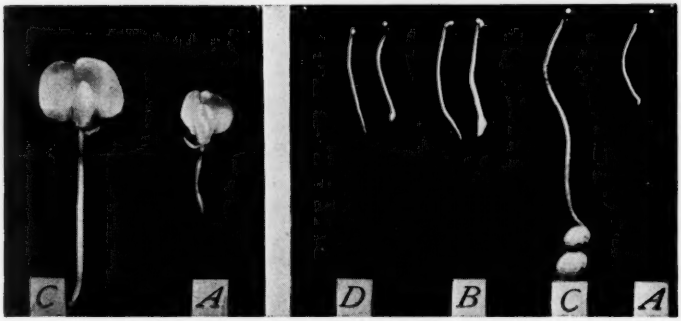

FIG. 1.—Flowers and gynophores produced in the several cultures: *C*, abundantly fruitful; *A*, very high nitrogen, non-fruitful; *D*, high carbohydrate; *B*, high nitrogen, limited fruitfulness.

dark house at 8:30 A.M. and back into it at 4:30 P.M., thus giving them an eight-hour day. Light intensities were greater than for series A, and were varied in a similar manner.

In this series the size of the flowers was used as an index to the nutritional level of the plants. It had been noticed that plants with a very limited accumulation of carbohydrates produced flowers about half as large as those on plants of only slightly greater carbohydrate reserve (fig. 1). Accordingly the nutrient solution and intensity of light were varied to maintain, as far as possible, the production of flowers of reduced size.

SERIES C: ABUNDANTLY FRUITFUL.—When the plants of this series were one month old, they were thinned to four per jar. Nutrient solution C-I was applied during the succeeding two and one-half

weeks, and solution C-II during the remainder of the experimental period. The concentration and quantity of nutrient solution were varied as necessary to maintain abundant production of flowers and gynophores. The plants were given full exposure to daylight.

SERIES D AND E: HIGH CARBOHYDRATE.—Both series were given the same treatment, except for slight variations noted hereafter. At the start of the treatments all nitrogenous salts were flushed from the sand with distilled water and nutrient solution DE. The concentrations of salts in this solution were very low, as ordinary concentrations had been found to be injurious and it was necessary to apply the solutions three times per week to stabilize the pH. Previous observations on high carbohydrate plants had shown that they soon accumulate ions to the point of toxicity, one of the most serious effects being the failure of such plants to accumulate any considerable amount of starch. When visible growth of the plants in these two series had practically ceased (June 9), the concentrations of all salts except KH_2PO_4 were reduced to one-half that recorded for them in table 4 in order to minimize possible injurious effects.

No nitrogen was added at any time to the nutrient solution of series E. Small amounts of nitrate were added to series D when it seemed that flower production was about to cease. Between June 22 and July 19, 8 liters of nutrient solution DE with $Ca(NO_3)_2$ at a concentration of 0.0001125 and 3 liters with this salt at a concentration of 0.000225 were added to each jar. Both series were exposed to the greatest available amount of direct sunlight.

In these series, as in series B, the size of the flowers was used as a criterion of nutritional level. About the time the lower leaves began to turn bright yellow and drop, the flowers became periodically much reduced in size and paler in color. Exfloration was discontinued in series D during the second cycle of floral reduction, and in series E during the third.

CONTROL OF PH.—On an average of once every two weeks, the pH of each jar in series B, C, D, and E was taken. At such times nutrient solution was applied to the cultures and the first 50 to 100 cc. of drip collected. All determinations were made by use of indicators on 2–3 cc. samples.

After determinations had been made, the pH was adjusted if nec-

essary with 0.001 N KOH or 0.006 N H_2SO_4. One or 2 liters of base or acid were added, followed by sufficient distilled water and nutrient solution to flush the sand. Table 5 summarizes the pH determinations.

It must be emphasized that each series was grown to secure a definite type response. Cultural treatment could not be a matter of inflexible routine therefore, for light, temperature, and relative humidity, which depend largely upon weather conditions, varied over a rather wide range. Accordingly it was necessary to modify cultural practice in order to produce the type responses desired.

TABLE 5

SUMMARY OF AVERAGE pH DETERMINATIONS

pH of nutrient solution	Series					
	Continuous application		Periodic application			
	A	AA	B	C	D	E
Applied	7.1	7.3	4.0	5.2	4.3	4.3
Drip	5.5	5.5	.....	.....	.....	.....
First drip before flushing	.....	.....	5.0	5.7	4.8	5.1
Mean deviation of jars	.....	.....	±0.43	±0.28	±0.60	±0.34

Comparative cultures of tomato.—For comparison a few tomato plants were included with each of the preceding treatments except AA. Two plants of the Marglobe variety were grown per jar, two jars having been included with series A and E and one with series B, C, and D. The number of plants used was limited.

Chemical methods

Harvest and preservation.—An abundance of sunshine favored the accumulation of carbohydrates toward the close of the experiment. Records of the weather bureau at the University of Chicago show that, for the eleven days preceding the first day of harvest, there was a daily average of 11.8 hours of sunshine.

Harvesting was done from July 19 to 21. In all cases the plants were placed in the dark house overnight. The following morning

each series was removed just before dissection, carefully flushed from the jars, and sand adhering to the roots and fruits dislodged with a jet of tap water.

Dissections were made at once in the laboratory. All series were separated into root, stem, petiole, leaflet, and composite fractions. Hypocotyls were included with roots. The stem fraction consisted of all stem tissue save that of the youngest 2 cm., the stem tips. Only healthy leaflets were used in the leaflet fraction, the remainder of such healthy leaves comprising the petiolar fraction. The composite fraction consisted of all leaves that were discolored in any way, stem tips, axillary buds, and (in the cases of all series except B and C) the gynophores. The fruits of series B were too small and few to constitute a separate fraction, and hence were combined with the gynophores to make a single fraction. The gynophore fraction of series C contained not only gynophores which had not reached the sand, but also those with very slightly enlarged ovaries. The fruit fraction of series C included both the fruits proper and the gynophores on which they had formed. Only the stems of the tomato plants were harvested.

The fractions were minced, preserved in 80 per cent alcohol by standard procedure, and stored in the dark. About two hours elapsed from the time series C was flushed from the jars until the fractions were put into alcohol. The period of harvest for the other series averaged about one hour. Extractions were made during the two succeeding weeks by standard procedure (**2**), except that Schleicher and Schüll filter paper no. 595 was used instead of hardened filter paper, to facilitate the rate of filtration. Filtrates were brought to volume and aliquoted at 25°C. All filtrates were free from cloudiness except that of the fruits in series C, which had a fine and somewhat stable suspension that appeared oily in nature. The ether extract of this filtrate appears in table 8. The residues of alcoholic extraction were ground first in a drug mill and then for twelve hours in a ball mill, dried in a vacuum oven at 80°, and stored in desiccators.

CARBOHYDRATES.—Copper precipitations were made by the QUISUMBING-THOMAS method (**16**) and the copper determined by the volumetric permanganate method of the A.O.A.C. (**1**).

SUGARS.—Sugar determinations were made on 500 cc. aliquots of filtrates prepared for analysis as described by STUART (**20**). Analyses were made for total sugars on 110 cc. of the cleared filtrates. The solutions were made just acid to methyl red with 10 per cent acetic acid. Two drops of Difco invertase solution were added and the reducing power was determined after three hours of enzymatic action at 37°. Total sugars are expressed as invert sugars. Reducing sugars were determined only on the stems.

STARCH AND DEXTRINS.—Beakers of 250 cc. capacity covered with sections of petri dishes were used for gelatinization and digestion. Samples of the dried residues were taken up with about 20 cc. of distilled water and gelatinized by heating on a steam bath and for 45 minutes in an 80° oven. After cooling, 4 cc. of fresh saliva was added and the samples digested for 45 minutes at 37°. The digested samples were heated over a steam bath, filtered hot, and washed free from sugars. Sufficient HCl was added to the filtrates to produce an acid concentration of 2.5 per cent, and the samples refluxed in boiling water for two and one-half hours. After cooling, they were made alkaline to methyl red but just acid to litmus paper. Results are expressed as starch, using 0.93 as the factor.

"HEMICELLULOSES."—The residues from which starch and dextrins had been removed were taken up in 2.5 per cent HCl, refluxed for two and one-half hours in boiling water, filtered, and cleared before analyses. Results are expressed as dextrose.

SOLUBLE NITROGEN.—Analyses were made on 100 cc. portions of the alcoholic extract by the reduced iron method (**15**).

INORGANIC NITROGEN.—The methods of STUART (**20**) and SESSIONS and SHIVE (**18**) were modified for the determination of ammonium and nitrate nitrogen. STUART'S method of preparation was used on 200 cc. aliquots of the alcoholic extract reduced to 50 cc. of cleared extract.

Ammonium nitrogen was determined as described by STUART, except that an aeration period of one and one-half hours was allowed. In addition, the lower ends of the foam traps of test-tubes containing the samples were closed with relatively loose cotton plugs to trap the fine alkaline spray formed during rapid aeration. Determinations checked much more closely when cotton plugs were used

than when they were omitted. This was especially true for the determinations of nitrate nitrogen.

For nitrate nitrogen, the method of SESSIONS and SHIVE was adapted to the Van Slyke-Cullen urea apparatus. Analyses were made on the ammonium-free aliquots. Two cc. of 10 per cent NaOH and about 0.3 gm. of deVarda's alloy were added and aeration carried on at a fairly rapid rate for six hours. In preliminary tests nitrate nitrogen had been quantitatively recovered by this method.

SOLUBLE ORGANIC NITROGEN.—This fraction was calculated as the difference between soluble nitrogen and inorganic nitrogen.

RESIDUAL NITROGEN. The micro-Kjeldahl method outlined by PREGL (**14**) was used for nitrogen analyses of the alcohol-extracted residues.

Results

VEGETATIVE AND REPRODUCTIVE RESPONSES

Comparative vegetative and reproductive responses of the several series are illustrated in figures 1 and 2. The plants within each series presented a uniform appearance when harvested.

SERIES A: VERY HIGH NITROGEN, NON-FRUITFUL.—The plants in this treatment were dark blue-green in color and grew slowly. The stems were slender and weak, producing few branches. During hot days the leaflets wilted considerably even though completely protected from direct sunlight. Many of the older leaves gradually died from too restricted carbohydrate synthesis. The accumulation of carbohydrates was so limited that eleven of the plants lost practically all their leaves and were removed from the experiment. A large proportion of the leaves on the remaining plants were partially discolored or dead at harvest, thus producing a rather large composite fraction. Root systems, observed at harvest, were markedly limited in extent and lacking in mechanical strength. Despite the extreme nutritional level at which this series was maintained, an average of about one small flower per plant was produced during the course of the experiment. During the period from June 17 to July 18 no flowers were removed. Of the eighteen flowers allowed to remain on the plants, only one gave rise to a gynophore.

SERIES AA: VERY HIGH NITROGEN, LIMITED FRUITFULNESS.—This series resembled series B in general appearance, but branched

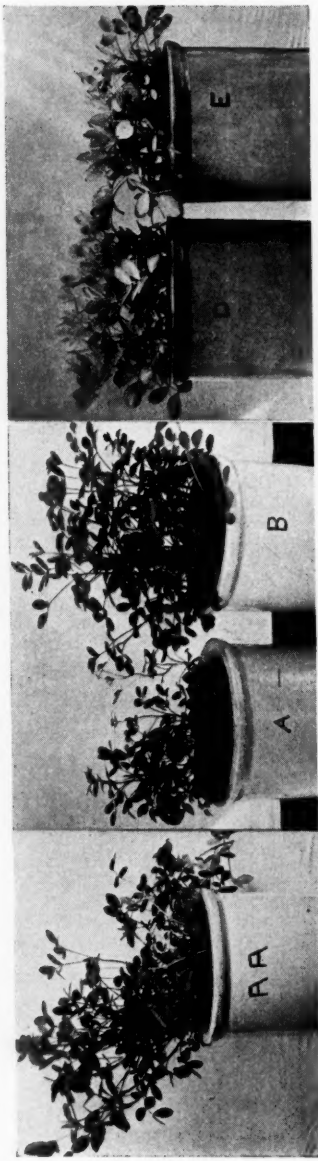

FIG. 2.—Plants in the several cultures employed: *AA*, very high nitrogen, limited fruitfulness; *A*, very high nitrogen, non-fruitful; *B*, high nitrogen, limited fruitfulness; *D*, *E*, high carbohydrate.

more freely. In reproductive responses the plants were intermediate between series A and B, producing an average of 9.6 flowers and 2.3 gynophores per plant.

SERIES B: HIGH NITROGEN, LIMITED FRUITFULNESS.—Plants of this series were much more vegetative than those of series A. From June 4, when exfloration was discontinued, they produced an average of 16.5 flowers, 7.6 gynophores, and 0.5 small fruits.

SERIES C: ABUNDANTLY FRUITFUL.—Both vegetative and reproductive responses were greatest in this series. The plants were larger, thicker stemmed than those in all other series, much branched, and almost as blue-green in color as those of the preceding series. They produced an average of 116.8 flowers, 19.6 gynophores, and 28.4 fruits.

SERIES D AND E.—Within two weeks after series D and E were flushed free from available nitrogen they had become conspicuously light green. A few days later the oldest leaflets became pale along their midribs and larger veins, remaining greenest at their margins. Shortly thereafter those slightly younger underwent

the same color change and the oldest ones turned bright yellow and dropped. Leaves formed after this time were few in number and uniformly pale green in color. In both series the diameters of the

TABLE 6

PERCENTAGE DRY MATTER

PLANT FRACTION	SERIES					
	A	AA	B	C	D	E
Roots	7.22	9.02	8.37	9.25	.....*	9.86
Stems	13.70	13.87	16.19	21.81	25.16	26.90
Petioles	10.23	10.56	12.37	17.20	17.92	18.58
Leaflets	13.33	14.04	15.53	17.38	18.28	18.71
Green gynophores	.....	.....	.....	10.53	.....	.....
Fruits	.....	.....	.....	31.60	.....	.....
Gynophores and fruits	.....	.....	16.30	.....	.....	.....
Composite	13.44	12.32	16.54	15.04	21.22	20.63
Tomato stems	6.17	.....	.....*	14.98	24.24	19.46

* Soluble solids were lost.

TABLE 7

PERCENTAGE SOLUBLE SOLIDS IN TOTAL SOLIDS

PLANT FRACTION	SERIES					
	A	AA	B	C	D	E
Roots	18.85	18.42	19.94	22.43	.....*	27.19
Stems	18.20	17.73	19.23	19.52	14.56	13.95
Petioles	31.19	30.07	29.36	25.99	21.20	20.16
Leaflets	30.89	27.52	27.56	27.78	26.21	26.62
Green gynophores	.....	.....	.....	27.37	.....	.....
Fruits	.....	.....	.....	14.42	.....	.....
Gynophores and fruits	.....	.....	29.23	.....	.....	.....
Composite	30.48	28.61	27.75	31.41	23.33	23.58
Tomato stems	39.68	.....	.....*	29.71	19.80	27.54

* Soluble solids were lost.

stems were intermediate between those of plants in series A and C. In general appearance and texture the plants were typically high carbohydrate.

TABLE 8

CARBOHYDRATE AND ETHER EXTRACT FRACTIONS (PERCENTAGE DRY WEIGHT)

PLANT FRACTION	CHEMICAL FRACTIONS	SERIES					
		A	AA	B	C	D	E
Roots	Total sugars	0.06	0.19	2.12	6.50	.....	14.43
	Starch and dextrins	2.94	2.56	2.68	1.96	(9.85)*	8.37
	Sub-totals	3.00	2.75	4.80	8.46	.....	22.80
	Hemicelluloses	13.15	13.46	12.81	11.20	(16.84)	11.40
	Totals	16.15	16.21	17.61	19.66	.....	34.20
Stems	Reducing sugars	None	None	0.20	2.34	1.82	1.35
	Total sugars	0.96	1.40	3.33	7.77	5.39	4.85
	Starch and dextrins	1.53	1.85	3.03	2.09	21.42	28.08
	Sub-totals	2.49	3.25	6.36	9.86	26.81	32.93
	Hemicelluloses	11.96	12.29	12.35	14.19	11.64	10.44
	Totals	14.45	15.54	18.71	24.05	38.45	43.37
Petioles	Total sugars	0.28	0.65	1.25	3.11	1.28	1.43
	Starch and dextrins	3.71	4.61	3.44	2.85	7.06	8.35
	Sub-totals	3.99	5.26	4.69	5.96	8.34	9.78
	Hemicelluloses	8.49	9.04	8.77	10.61	10.52	11.18
	Totals	12.48	14.30	13.46	16.57	18.86	20.96
Leaflets	Total sugars	0.02	0.17	0.34	1.15	0.85	1.40
	Starch and dextrins	1.14	1.51	1.93	4.40	13.42	10.66
	Sub-totals	1.16	1.68	2.27	5.55	14.27	12.06
	Hemicelluloses	4.28	4.38	4.19	5.20	6.41	5.97
	Totals	5.44	6.06	6.46	10.75	20.68	18.03
Gynophores and fruits	Total sugars	.....	.....	5.55	.....	.....	.....
	Starch and dextrins	.....	.....	8.88	.....	.....	.....
	Sub-total	.....	.....	14.43	.....	.....	.....
	Hemicelluloses	.....	.....	7.03	.....	.....	.....
	Total	.....	.....	21.46	.....	.....	.....
	Ether extract	.....	.....	6.28	.....	.....	.....
Green gynophores	Total sugars	.....	.....	.....	2.82	.....	.....
	Starch and dextrins	.....	.....	.....	6.04	.....	.....
	Sub-total	.....	.....	.....	8.86	.....	.....
	Hemicelluloses	.....	.....	.....	9.42	.....	.....
	Total	.....	.....	.....	18.28	.....	.....
	Ether extract	.....	.....	.....	1.63	.....	.....
Fruits	Total sugars	.....	.....	.....	6.47	.....	.....
	Starch and dextrins	.....	.....	.....	6.89	.....	.....
	Sub-total	.....	.....	.....	13.36	.....	.....
	Hemicelluloses	.....	.....	.....	4.69	.....	.....
	Total	.....	.....	.....	18.05	.....	.....
	Ether extract	.....	.....	.....	38.56†	.....	.....

* Percentages in parentheses are calculated on basis of residues of alcoholic extraction.

† Alcoholic soluble extract, 2.95%; alcoholic insoluble extract, 35.61%.

TABLE 8—*Continued*

Plant fraction	Chemical fractions	Series					
		A	AA	B	C	D	E
Composite	Total sugars	0.02	0.75	1.10	2.21	2.05	2.14
	Starch and dextrins	1.85	2.74	2.85	5.90	13.72	14.45
	Sub-totals	1.87	3.49	3.95	8.11	15.77	16.59
	Hemicelluloses	5.70	5.85	6.44	7.56	7.48	5.54
	Totals	7.57	9.34	10.39	15.67	23.25	22.13
Tomato stems	Total sugars	0.85	.....	.....	20.46	12.34	17.70
	Starch and dextrins	3.15	.....	(3.22)	9.24	15.76	21.55
	Sub-totals	4.00	.....	.....	29.70	28.10	39.25
	Hemicelluloses	8.68	.....	(15.83)	11.75	13.18	11.94
	Totals	12.68	.....	.....	41.45	41.28	51.19

SERIES D: HIGH CARBOHYDRATE, GYNOPHORE-PRODUCING.—These plants were identical in appearance with those of series E until about eight days after the first addition of $Ca(NO_3)_2$ to their nutrient solution. During the remainder of the experiment, the first three weeks of July, a few new leaves appeared and the plants as a whole became slightly greener. No nodules were visible at harvest on any of the high carbohydrate plants, except for a few small ones near the hypocotyl of a single plant.

For the period during which flowers were no longer removed until the application of $Ca(NO_3)_2$ caused an increase in blooming (June 6 to July 1), the plants produced an average of 6.8 flowers; during the remainder of growth (July 2–18) the average was 10.3 flowers. Only one gynophore had been produced by June 18, a total of twenty by July 4, and of twenty-three by July 20 by the forty-eight plants in this series. Enlargement of ovaries was scarcely perceptible.

SERIES E: VERY HIGH CARBOHYDRATE, NON-FRUITFUL.—From July 5 to 18 no blossoms were removed from the plants of this series. An average of 4.1 flowers was produced per plant. Only one plant in the entire lot developed a gynophore.

COMPARATIVE CULTURES OF TOMATO.—Although the vegetative responses of tomatoes were similar to those of peanuts, reproductive behavior was quite different. Tomato plants of series A were moderately vegetative, medium green in color, and very succulent. During the period of much reduced illumination in the earlier part of

TABLE 9

NITROGEN FRACTIONS (PERCENTAGE DRY WEIGHT)

PLANT FRACTION	CHEMICAL FRACTIONS	SERIES					
		A	AA	B	C	D	E
Roots	Ammonium N	0.113	0.276	0.035	0.025	.....	None
	Nitrate N	0.130	0.190	0.310	0.040	.....	None
	Soluble organic N	0.673	0.395	0.218	0.146	.....	0.007
	Residual N	1.913	1.880	1.571	1.638	(1.003)	0.886
	Total N	2.829	2.741	2.134	1.849	.....	0.893
Stems	Ammonium N	0.067	0.049	0.025	0.009	None	None
	Nitrate N	0.470	0.610	1.010	0.060	None	None
	Soluble organic N	1.086	0.568	0.233	0.087	0.069	0.065
	Residual N	1.143	1.102	1.022	0.645	0.357	0.326
	Total N	2.766	2.329	2.290	0.801	0.426	0.391
Petioles	Ammonium N	0.096	0.048	0.042	0.027	None	None
	Nitrate N	0.990	1.120	1.060	0.110	None	None
	Soluble organic N	1.240	0.490	0.817	0.096	0.117	0.094
	Residual N	1.845	1.599	1.606	0.874	0.602	0.555
	Total N	4.171	3.257	3.525	1.107	0.719	0.649
Leaflets	Ammonium N	0.100	0.049	0.046	.....	None	None
	Nitrate N	0.280	0.310	0.460	0.367	None	None
	Soluble organic N	1.320	0.563	0.629	.....	0.195	0.179
	Residual N	4.717	4.447	4.426	3.130	1.114	1.205
	Total N	6.417	5.369	5.561	3.497	1.309	1.384
Gynophores and fruits	Ammonium N	.....	.....	0.010	.....	.....	.....
	Nitrate N	.....	.....	0.390	.....	.....	.....
	Soluble organic N	.....	.....	1.188	.....	.....	.....
	Residual N	.....	.....	2.839	.....	.....	.....
	Total N	.....	.....	4.411	.....	.....	.....
Green gynophores	Ammonium N	.....	.....	.....	0.030	.....	.....
	Nitrate N	.....	.....	.....	0.110	.....	.....
	Soluble organic N	.....	.....	.....	1.143	.....	.....
	Residual N	.....	.....	.....	1.721	.....	.....
	Total N	.....	.....	.....	3.004	.....	.....
Fruits	Ammonium N	.....	.....	.....	0.015	.....	.....
	Nitrate N	.....	.....	.....	0.020	.....	.....
	Soluble organic N	.....	.....	.....	0.160	.....	.....
	Residual N	.....	.....	.....	2.327	.....	.....
	Total N	.....	.....	.....	2.522	.....	.....
Composite	Ammonium N	0.174	0.073	0.055	0.044	None	None
	Nitrate N	0.290	0.280	0.720	0.100	None	None
	Soluble organic N	1.470	0.763	0.517	0.367	0.257	0.221
	Residual N	3.162	3.033	2.760	2.912	1.127	1.034
	Total N	5.096	4.149	4.824	3.423	1.384	1.255

TABLE 9—*Continued*

Plant fraction	Chemical fractions	Series					
		A	AA	B	C	D	E
Tomato stems	Ammonium N	1.259	.....	.....	0.262	None	None
	Nitrate N	0.820	.....	.....	0.040	None	None
	Soluble organic N	2.116	.....	.....	0.707	0.201	0.221
	Residual N	1.163	.....	(1.258)	0.853	0.500	0.421
	Total N	5.358	.....	.....	1.862	0.701	0.642

the experiment, they developed two to three flower buds per cluster, which remained very small and eventually dried and fell off. Later light intensity was slightly increased and the buds formed were larger and persistent. The series B plants were vigorously vegetative, dark green in color, succulent, and but sparingly branched. They produced clusters of large flowers which fell shortly after opening. Even though the plants of series C did not grow so tall as those of series B, they were profusely branched and bore a relative abundance of flowers and fruits. Series D and E were almost identical in general appearance, growing very little and becoming yellowish and woody. The flower buds of series D, however, were slightly larger than those of series E. One plant of series E blossomed, bore one small fruit and died.

Chemical data

Data of percentage dry matter are given in table 6, of percentage soluble solids in total solids in table 7, of carbohydrate and ether extract analyses in table 8, and of nitrogen fractions in table 9.

Discussion

Flower-to-gynophore interval

It is apparent that the enlargement of fruits noticeably increased the average flower-to-gynophore interval but did not change the mode of this interval.

In certain preliminary experiments it had been observed that the average time required for the appearance of the first gynophore on a plant was about one week after its flower had opened. The enlargement of fruits, however, was found to increase the average interval

approximately 50 per cent, as shown by the data for July 16 to 31 in table 1. The records in this table are for plants which had commenced to bloom about June 14, so that fruit enlargement was in progress when the first flowers were tagged for a study of the interval. The period between the opening of flowers and the appearance of gynophores became shorter again during August, because most of the gynophores produced during July and August arose from stems which drooped over the rims of the jars and consequently did not set fruits.

When the data are analyzed on the basis of statistical modes, there appears to be a much greater degree of stability for the period between anthesis and the initiation of meristematic activity in the gynophore than the data of averages indicate. Table 1 shows that (1) within each series the statistical mode of this period was not altered by the onset of fruit development, having maintained itself at seven days in the high nitrogen series and at nine days in each of the other three series throughout the period of observation; and (2) with the possible exception of the high nitrogen series, there was no correlation between the mode of the interval and the amount of nitrate supplied.

BEHAVIOR OF VERY LOW NITROGEN PLANTS.—From the preceding comments on modal stability, it is evident that, for plants producing gynophores, the general level of nutrition has but little influence on the rate of their appearance. If very low nitrogen plants bearing gynophores and fruits have their supply of available nitrogen sufficiently reduced, however, the nutritional level of such plants will fall below the threshold of meristematic activity in the gynophore initials. This change occurred in the very low nitrogen plants as recorded in table 1. Until August 1 they produced gynophores with a modal frequency equal to that of the medium nitrogen series; but after August 1 they developed no gynophores at all, although they continued to bloom sparingly.

The response of the plants in series D is also significant in this respect. They were below the threshold of gynophore production when the application of minute quantities of nitrate was started on June 22. By July 4 the forty-eight plants in the series had produced a total of twenty gynophores; but only three additional gynophores

had formed by July 20, although the intermittent application of nitrate was continued until that date. These responses indicate that the nutritional level of most of the plants was raised above the threshold of gynophore initiation for a period of not more than twelve days (from June 22 to July 4), and that the extension of gynophores soon caused the nutritional level to drop again below that threshold.

PRECOCITY OF GYNOPHORE DEVELOPMENT.—Correlation of the preceding observations indicates a definite precocity of gynophore development for plants representing a wide range of nutritional levels; that is, if gynophores are formed at all, the modal frequency of their flower-to-gynophore intervals will be confined to a very narrow range not exceeding a period of nine days.

Lack of sufficient data makes it impossible to extend the application of this concept to very high nitrogen plants such as those of series A and B.

RELATIVE REPRODUCTIVITY OF SERIES B AND C

The data given under "Vegetative and reproductive responses" for the fruiting behavior of series B are apt to be misleading, as they are based on the numbers of gynophores and fruits. Data comparing average weights of these plant parts with those of series C are significant. On a green weight basis the average weights of non-fruiting (green) gynophores in series B and C were 0.06 and 0.13 gm. respectively, and of fruits 0.4 and 1.02 gm. As each plant of series B produced an average of only 0.2 gm. of fruit and each one of series C an average of 29.02 gm., the former may be considered relatively non-fruitful.

STABILITY OF REPRODUCTIVE FUNCTION

In this discussion of stability emphasis is placed on the opening of flower buds and the initial stages of fruit formation. These two phases of reproduction are less readily modified in the peanut plant than in the tomato, and may be summarized as follows. In series A (very high nitrogen) the peanut bloomed sparingly and was non-fruitful, but the tomato failed even to open flower buds. In series B (high nitrogen) the peanut produced many flowers and a few small fruits, while the tomato blossomed and remained entirely non-fruitful. In series D (high carbohydrate) the peanut bore numerous

flowers and a few gynophores, but the tomato gave rise to flower buds only. In series E (very high carbohydrate) the peanut blossomed freely and was non-fruitful, while reproduction in the tomato was limited to the formation of a few small flower buds. These results show that the reproductive function is more readily suppressed in the tomato than in the peanut.

General trends of chemical data

Table 6 shows a consistent increase in the percentage dry matter from highest nitrogen to highest carbohydrate series for all plant fractions except the composites of series A and B. The latter are relatively high in percentage dry matter, largely because the leaflets included had a higher proportion of discolored and partially desiccated tissue.

The percentage of soluble solids in total solids tends to decrease from highest nitrogen to highest carbohydrate series, with the notable exception of the root fractions, which have an inverse correlation.

The following trends are evident in tables 8 and 9. From highest nitrogen to highest carbohydrate series soluble and residual nitrogen decrease and total carbohydrates increase. These trends agree with those of investigations on other species not conditioned by photoperiodic behavior or by specific temperature treatments.

There is no uniformity of opinion as regards the relative importance of the several carbohydrate and nitrogen fractions in their relations to vegetation and reproduction of plants (**5, 8**). Lack of concordance in viewpoints is in part associated with differences inherent in the plants which have been studied. Some of them are normally annual; many are herbaceous but potentially perennial; others are woody perennials which have alternate periods of dormancy and growth (**13, 20**), and which may be subject to cumulative effects at present undefined. In photoperiodic response they represent short day plants, indeterminate bloomers, and those which flower only under long day conditions (**10**). Many plants are characterized by asexual storage organs, the enlargement of which may modify the interpretation of chemical data. Certain plants are highly sensitive to range of temperature (**11, 24, 25**). Because of these and other in-

herent differences, it is to be anticipated that the vegetative and reproductive responses will not always be correlated with exactly the same chemical fractions.

Most of the factors specifically mentioned in the preceding discussion were not limiting in the present study of the peanut. It is neither an annual plant nor one sufficiently hardy to exhibit the growth rhythm of a woody perennial, but continues to fruit indefinitely under certain conditions. It has proved to be an indeterminate bloomer. None of its asexual parts become especially enlarged for food storage. When given an adequate supply of water and mineral salts, it grew luxuriantly under the conditions of temperature prevailing during the period of experimentation.

The significant chemical fractions in the peanut plant are considered to be (1) total sugars, starch, and dextrins taken as a unit; and (2) total nitrogen.

CARBOHYDRATE FRACTIONS.—In correlating chemical data in nutritional studies, such as the one reported here, it seems logical to include only those chemical fractions which correspond to the vegetative and reproductive responses obtained. On this basis total sugars, starch, and dextrins (table 8) considered together are the significant carbohydrates; for neither total sugars alone nor starch and dextrins alone are consistently correlated with the responses observed. Although "hemicelluloses" were determined on all plant fractions, their ranges in percentage from highest to lowest nitrogen series were much too small to regard this chemical fraction of any importance in the peanut.

NITROGEN FRACTIONS.—The choice of significant nitrogen fractions was not restricted to the preceding basis of correlation. Although the determinations for residual and soluble organic nitrogen (table 9) correspond, on the whole, to the nutritional levels of their respective cultural treatments, those of the inorganic forms do not. On the basis of correlation alone, only the assimilated nitrogen should be used for interpretive purposes. The inorganic fractions are included in the interpretation presented because they aid in maintaining metabolic processes at approximately a given level. A common case in point is that of a medium nitrogen plant growing under conditions favorable to carbohydrate synthesis. So long as such a

plant is given an adequate supply of inorganic nitrogen its nutritional level will undergo little change. But if the plant is suddenly deprived of all externally available nitrogen, it soon becomes high carbohydrate. A progressive change in its nutritional level occurs as the inorganic nitrogen in its tissues becomes metabolized without being replaced by absorption from the soil solution. Even though the inorganic nitrogen fractions in plant tissues may at times be present in excess of immediate needs for maintenance of a nutritional level, at least part of such nitrogen functions in this manner in intermediate bloomers.

The influence of inorganic nitrogen in plant tissues on the principal phases of carbohydrate metabolism is a relatively new field of investigation. HAMNER (3) has recently reported that nitrates have a direct influence on the rate of respiration in wheat. The effects of inorganic nitrogen on photosynthesis need careful study.

In view of the preceding contingencies, total nitrogen, rather than any specific nitrogen fraction, should be used in correlating nutritional studies of the peanut and of other plants of similar physiological constitution.

INTER-SPECIAL CORRELATION OF CHEMICAL DATA.—When the analyses for tomato and peanut stems from any specific cultural treatment are compared, two correlations appear. Data in table 9 show that, within each cultural treatment, the tomato maintained approximately twice the concentration of total nitrogen found in the peanut. On the other hand, no such consistent inter-special correlation exists in the data for carbohydrates (table 8). Accordingly when the two species are considered together, nutritional levels are more closely associated with the nitrogen than with the carbohydrate fraction.

CORRELATION OF VEGETATION AND REPRODUCTION

In the five main cultural treatments, vegetative activity increased from very high nitrogen to abundantly fruitful plants and decreased from the latter to very high carbohydrate plants. Gynophore and fruit production followed similar quantitative changes. The setting and enlargement of fruits did not obviously retard the rate of vegetative extension of the fruitful plants in series C. For the peanut,

then, vegetation and reproduction are not opposing tendencies, but are complements in the course of development. This is true also for the tomato (**5**).

Miscellaneous observations

A few grams of coarsely ground seed of the high and very low nitrogen series of 1933 have been kept in 250 cc. bottles since the time of harvest. In less than a year the high nitrogen sample had become rancid in odor and taste, but the very low nitrogen sample was still free from rancidity after a period of three years. The seed of the high nitrogen series (1933) had slightly less ether extract than that of the very low nitrogen series (table 3).

A distinct gradation of pigmentation in seed coats was observed throughout the four series grown in 1933. The seed coat color of the highest nitrogen series was the usual rich brown, that of the very low nitrogen culture was pale and almost pink in color. If the color of the seed coat is to be employed in the classification of varieties (**19**), it will be necessary to grow all the plants under the same environmental conditions.

As the plants in series D and E approached the high carbohydrate condition, the new leaflets were observed to have numerous marginal hairs or trichomes. At the time most of these plants were harvested, a jar of six plants was given an abundance of available nitrogen and placed in the shade. Leaf primordia formed after the resumption of growth produced smooth-margined leaflets only. The test was repeated on other high carbohydrate plants with the same results. The culture of six plants of series E was modified by giving them an excess of the chloride ion for a period of forty days. One result of this modification was the almost complete disappearance of starch from the stems. When these plants were abundantly supplied with nitrogen even the first leaflets to expand were free from marginal ciliation. Leaflets of high nitrogen plants (series A, AA, and B) were smooth-margined, and those of series C bore scattered hairs much less conspicuous than those of series D and E. These results indicate that the growth of marginal hairs is associated with a relatively high carbohydrate level of metabolism.

Summary

1. In the peanut plant, greatest vegetative extension and fruitfulness occurred in the same cultural treatment.

2. All high nitrogen plants were dark blue-green in color, slender stemmed and succulent; all high carbohydrate plants were very pale yellow-green, relatively thick stemmed, and firm in texture. Both were weakly vegetative and non-fruitful.

3. From highest nitrogen to highest carbohydrate series the percentage dry matter and total carbohydrates increased, and the percentage soluble solids and total nitrogen decreased consistently with vegetative and reproductive responses. The root fractions increased in percentage soluble solids.

4. "Hemicelluloses" were not correlated with levels of nutrition.

5. The high carbohydrate condition favored a slightly greater percentage of ether extract in the seeds and gynophores, the suppression of rancidity in the seeds, and the development of marginal hairs on the leaflets.

6. Gynophore development was precocious. The mode of the flower-to-gynophore interval was only slightly influenced by the enlargement of fruits on older gynophores or by extensive variations in metabolic levels, but elongation of the gynophores was abruptly inhibited by extreme levels of nutrition.

7. The fruiting tendency was less sensitive to nutritional change in the peanut than in the tomato.

8. The concentration of the phosphate ion in nutrient solutions must be greatly reduced to obviate injury when the peanut plant is grown in the light.

9. Ammonium nitrogen nutrient solutions which do not precipitate when applied at pH 7.1 are reported.

10. A method for determining nitrates by a six-hour aeration period is described.

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PUERTO RICO EXPERIMENT STATION
U.S. DEPARTMENT OF AGRICULTURE
MAYAGÜEZ, PUERTO RICO

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HISTOLOGICAL AND MICROCHEMICAL STUDIES OF THE REACTIONS OF TOMATO PLANTS TO INDOLEACETIC ACID

H. A. BORTHWICK,[1] K. C. HAMNER,[2] AND M. W. PARKER[3]

(WITH TWENTY FIGURES)

Introduction

Histological studies of bean plants which had been treated with indoleacetic acid were recently made at the United States Horticultural Field Station at Beltsville, Maryland, and at the University of Chicago (1). These results made it desirable that similar work be done with other plants that respond to indoleacetic acid. The tomato, which has been used extensively as a test plant in physiological studies on the effects of growth promoting substances, was chosen as a second subject for histological study. A comparison of the histological responses of tomato with those of bean is particularly desirable because of the fundamental difference in arrangement of tissues in the two plants, the tomato stem having an internal phloem while the bean stem does not.

MATERIAL AND METHODS.—Seedlings of the Pritchard variety were grown during the fall and winter months in the greenhouse at the United States Horticultural Field Station. They were started in flats and transplanted to small pots, from which they were successively shifted to larger ones as their size demanded. They were subjected to the natural light intensity and day length prevailing in the latitude of Beltsville. Environmental conditions were such that good growth was maintained.

A mixture of 20 mg. of indoleacetic acid in 1 gm. of lanolin was applied to the cut surface of stems decapitated in the mid portion of the internode above the second lobed leaf when the plants were expanding their fourth lobed leaf. Differentiation of the primary tissues of the internode treated was definite, but little or no secondary

[1] Morphologist, [2]Associate Physiologist, [3]Associate Physiologist, all at the U.S. Horticultural Field Station, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U.S. Department of Agriculture, Beltsville, Md.

activity was apparent. Additional plants were decapitated and kept as controls, pure lanolin being smeared over the cut surface of some, and the rest being left with the cut surfaces untreated.

Specimens were collected at intervals of 6 hours for the first 72 hours and every 12 hours thereafter until 216 hours had elapsed. Material for histological purposes was fixed in Navashin's solution and imbedded by the butyl alcohol-paraffin method. Sections were cut at 10 μ and stained with the triple stain. Comparable samples were examined microchemically immediately after each collection.

Gross responses to treatment

The first change to be observed after application of the indoleacetic acid mixture to the stem is usually an epinastic movement of one or more leaves next below the point of application. This frequently takes place within a few hours after treatment. There is usually no bending of the stem but there is a loss of color for a distance of 3 to 5 mm. below the cut surface. At the end of about two days the upper part of the stem shows evidence of enlargement and further loss of color. By the end of a week small bumps at the periphery of the swollen region mark the positions of adventitious roots, which emerge under favorable conditions. The portion of the internode below the swollen area retains its natural color and continues to grow, for a time at least, at approximately the same rate as the internode just below it. This same region of the controls, however, whether treated with pure lanolin or left untreated, fails to continue to enlarge and becomes woody.

The indoleacetic acid does not inhibit the development of buds below the treated area. In the plants treated terminally and in their controls these buds appeared to start growth immediately, and in a short time the uppermost one or two of them assumed the lead and produced vigorous branches (fig. 1*A*).

Histological observations

The epidermal cells covering the area of greatest activity show no striking response to indoleacetic acid other than a slight enlargement. They are stretched and sometimes ruptured by the expansion of tissues inclosed by them.

The cortex of an untreated stem consists of four regions: an outer layer of chlorophyll bearing cells, a zone of collenchyma, a layer of parenchyma, and an endodermis. The chlorophyll bearing cells are small and thin walled, with extensive intercellular spaces. In treated stems these cells begin to increase in size within 24 hours, and even-

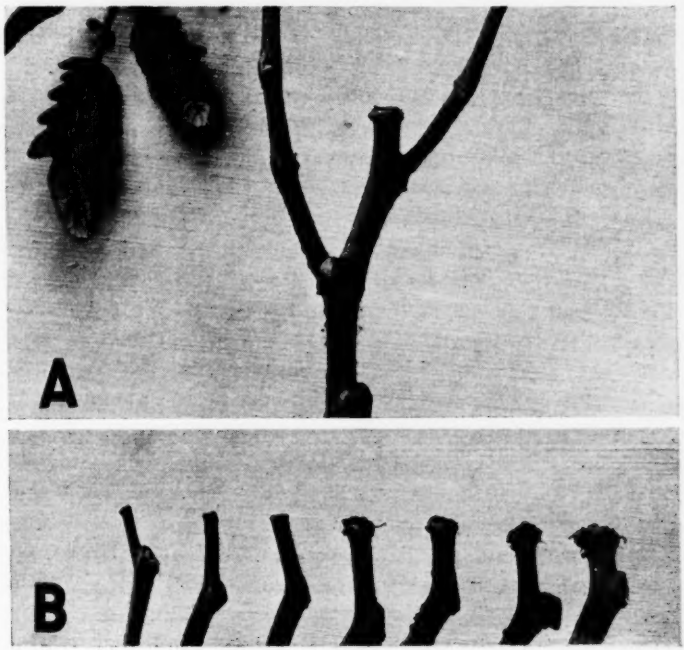

FIG. 1.—*A*, 10 days after decapitation of stem at center and treatment of same with indoleacetic acid-lanolin mixture. Strong lateral growths have developed from lower nodes. *B*, at left three stems decapitated and treated with lanolin only; at right four stems treated with indoleacetic acid mixture. All 10 days after treatment.

tually their radial diameter becomes two or three times that of those untreated. The walls remain thin and the intercellular spaces become less prominent. The chloroplasts become much less evident than in untreated material, which accounts for the paler green color of the treated areas.

The collenchyma of the cortex of untreated stems occurs as a

continuous layer two to five cells thick (fig. 2). Its cells are approximately isodiametric in cross section and their length is ten to fifteen times their diameter. Following treatment applied to a terminally cut surface, the collenchyma cells enlarge radially within the first 24 hours (fig. 3). At the end of about 54 hours, divisions in these cells in the upper 1 or 2 mm. of stem become abundant. These divisions are seen much more frequently in longitudinal than in cross sections, although they are by no means uncommon in the latter.

Centripetal to the collenchyma there are one to three layers of parenchyma. In treated stems these cells enlarge greatly, and about 54 hours after treatment they are in a very active state of division, the planes of division occurring in any direction (fig. 4).

Approximately 30 hours after treatment the cells of the endodermis begin to undergo tangential division, and by 54 hours divisions are very frequent and in all planes. The activity in the endodermis is much more pronounced than in the rest of the cortex, and this results in the formation of a band of highly meristematic tissue two to five cells wide. In the region where adventitious roots are formed, parts of this band of endodermal tissue are pushed out as coverings over the tips of the roots.

The cells of the pericycle which would normally mature as fiber cells fail to respond markedly to applications of the indoleacetic acid mixture, although after treatment they may enlarge somewhat and the walls of the potential fibers may thicken slightly. Many of the parenchymatous cells are apparently crushed by the activity of the phloem and endodermal tissues adjacent to them (figs. 10, 11). Some of those which were embryonic at the time the application was made do enlarge and undergo several divisions, but the derivatives of these play a relatively unimportant part in the development of the tumor or in the formation of adventitious roots.

The parenchyma of the external phloem exhibits a marked sensitivity to applications of the indoleacetic acid mixture. The tissues immediately adjacent to the treated surface show little change, even after a number of days (fig. 9*A*). Within 54 hours, however, the parenchymatous cells of the phloem lying within 2 mm. of the treated surface begin active divisions in all planes (fig. 4*A*). It is usually

from some of the derivatives of these cells that the external adventitious roots eventually become differentiated (fig. 11*A*, *B*). Other cells may differentiate as large tracheids, strands of sieve tubes and companion cells, or they may remain meristematic. In these experiments the activity in the external phloem did not extend more than 3 mm. from the treated surface, and the greatest activity occurs between 0.6 and 2.0 mm.

The internal phloem is as sensitive in its response to the treatment as is the external. There is little or no activity immediately adjacent to the cut surface, but within 54 hours those parenchymatous cells which lie about 1.0 mm. below the treated surface become very active (fig. 4); and later, as cell divisions continue, it is sometimes difficult to distinguish their derivatives from those of adjacent, actively dividing pith cells (fig. 8). Derivatives of these internal phloem parenchyma cells often differentiate as tracheids. Activity in the internal phloem extends farther down the stem as a result of the treatment than does the activity in any other tissue. Certain of the parenchymatous cells at levels as distant as 4 mm. from the treated surface undergo a few divisions and tracheids are derived from them.

The xylem parenchyma becomes relatively active as a result of treatment and this results in lateral displacement of many of the conducting elements. This activity is confined to portions of the stem 0.25 to 2.5 mm. from the treated surface.

Within 54 hours after treatment, the cells of the pith adjacent to the protoxylem points show considerable activity, and shortly thereafter many dividing cells are apparent throughout the pith region at distances of 0.5 to 2.0 mm. from the treated surface. The pith as a whole does not become meristematic beyond these limits, and most of the cells in the meristematic region undergo only a few divisions.

At the time of the treatment, certain of the pith cells adjacent to the sieve tubes and companion cells of the internal phloem had started differentiation as internal fibers. These cells do not become meristematic as a result of the treatment, and continue at a slow rate their differentiation as fibers.

The cells of the pith and rays adjacent to the primary xylem become relatively more active, and the activity extends farther from

the cut surface than is true for the pith as a whole. Those cells lying centripetally to the internal phloem become very active (figs. 8*B*, 9*B*); and it is from these derivatives, together with the derivatives of the internal phloem region, that the internal adventitious roots are differentiated.

Microchemical observations

Methods

Nitrates.—Diphenylamine reagent (0.1 gm. diphenylamine in 10 ml. of 75 per cent H_2SO_4) was applied to fresh sections. Observations were made immediately and the intensity of the blue color that developed in various sections was made a basis of comparison.

Protein.—Millon's reagent was applied to the sections. Immediately after slight heating, observation of the red precipitate was made.

Starch.—Sections were treated with iodine potassium iodide (0.3 gm. I, 1.5 gm. KI, diluted to 100 ml.).

Results

Nitrates.—The concentration of nitrates diminished during the experiment in both the treated and the untreated stems. However, there was more nitrate in the control. As the stem treated with the indoleacetic acid mixture began to swell, the nitrates disappeared entirely from the swollen area but were often abundant in the tissues just below.

Protein.—The protein content of the treated stem increased in those areas where cell divisions became most abundant, while the protein content of the control stem remained rather constant during the experiment. Tissues of the stem in which marked meristematic activity subsequently occurred could readily be localized by an increase in the protein concentration, even before any cell divisions took place.

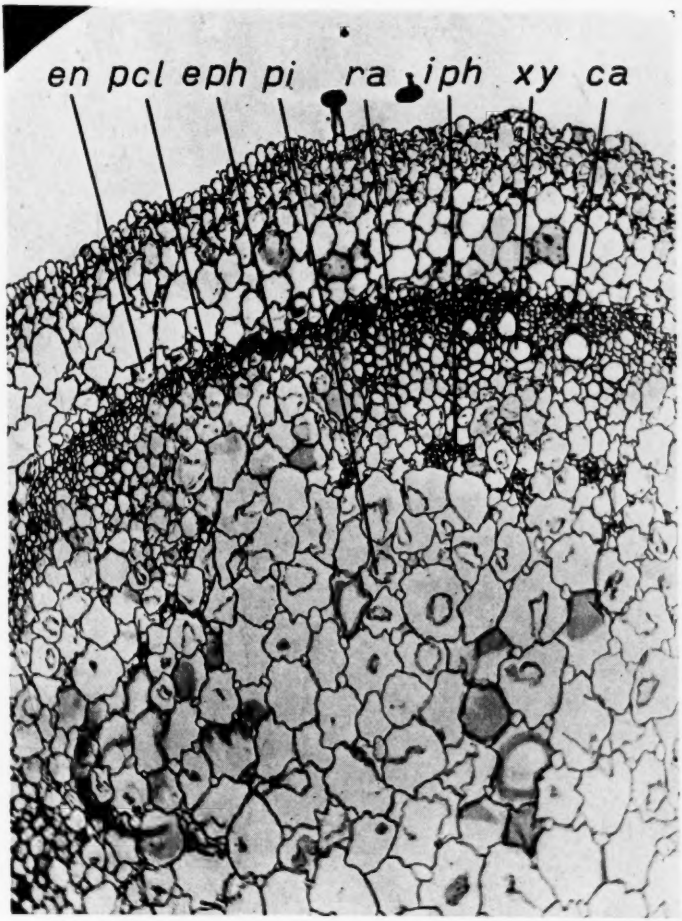

FIG. 2.—Transection through middle of third internode of tomato stem at time of application of indoleacetic acid, showing stages of development of various tissues. *en*, endodermis; *pcl*, pericycle; *eph*, external phloem; *iph*, internal phloem; *ca*, cambium; *xy*, xylem; *ra*, ray; *pi*, pith.

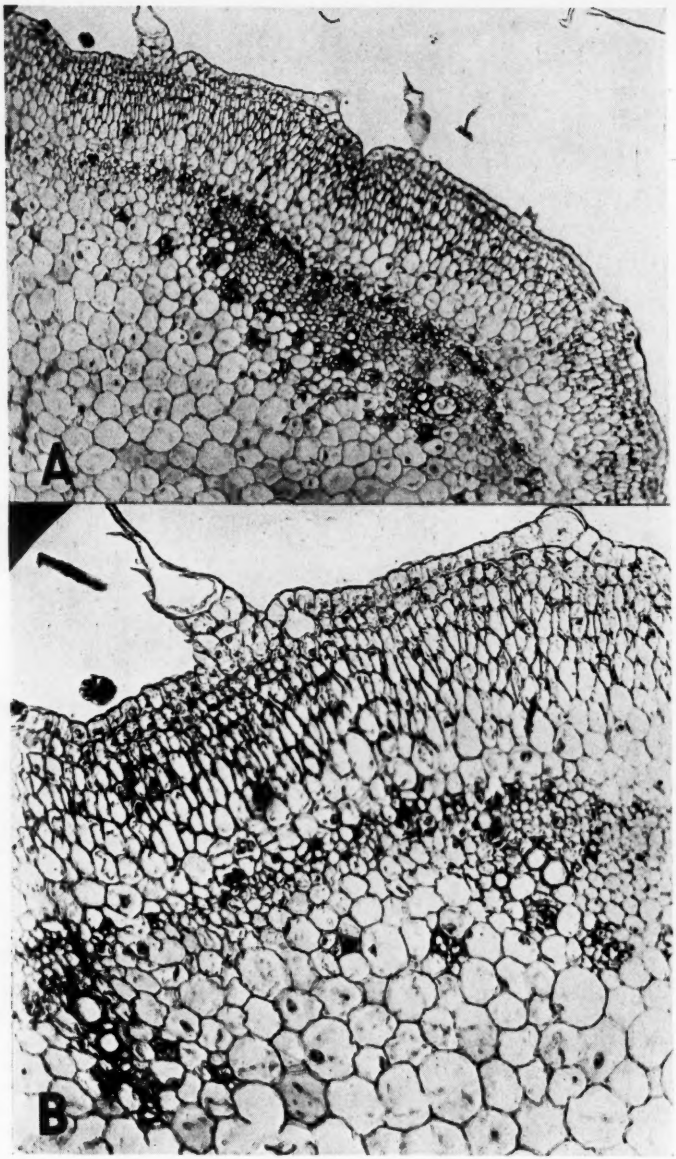

FIG. 3.—Twenty-four hours after treatment. *A*, section 400 μ from cut surface; *B*, sector of same enlarged. Epidermal cells slightly enlarged; not much division. Outer cortical cells appreciably enlarged and elongated radially. Endodermal cells enlarged. Other tissues show slight change.

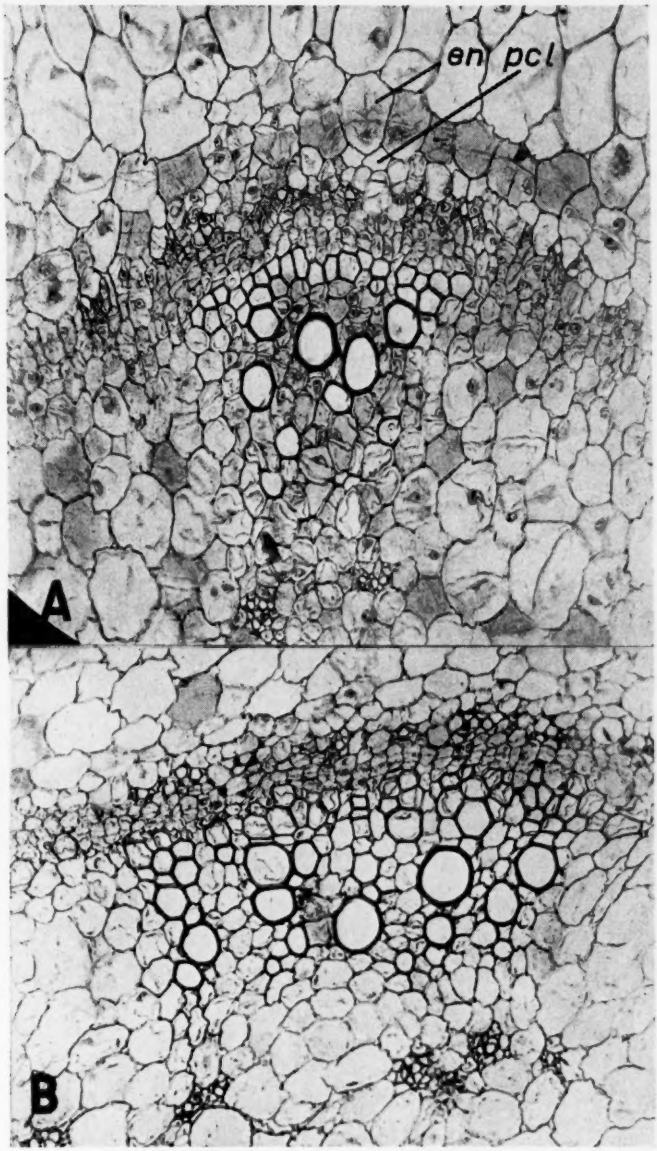

FIG. 4.—Fifty-four hours after treatment. *A*, section of bundle about 1.5 mm. below cut surface. Endodermal cells divided tangentially and radially. Occasional divisions in other cortical cells but not in pericycle. Parenchymatous cells in primary xylem, pith, and near internal phloem actively dividing. Divisions in external phloem frequent and in all planes. Slight differentiation of tissues from the cambium. *B*, 3 mm. below cut surface. No divisions in any tissue except the cambium.

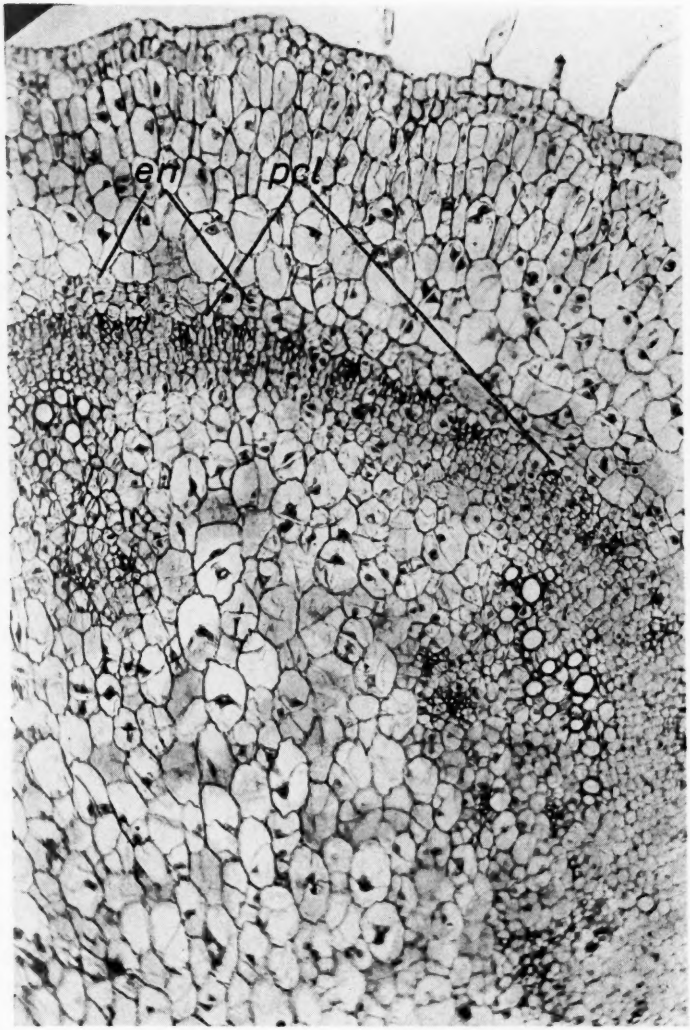

FIG. 5.—Sixty hours after treatment; about 1 mm. from cut surface. Much activity in cortical parenchyma, endodermis, external and internal phloem parenchyma, xylem parenchyma, ray, and pith. Pericyclic fibers unchanged except more nearly mature.

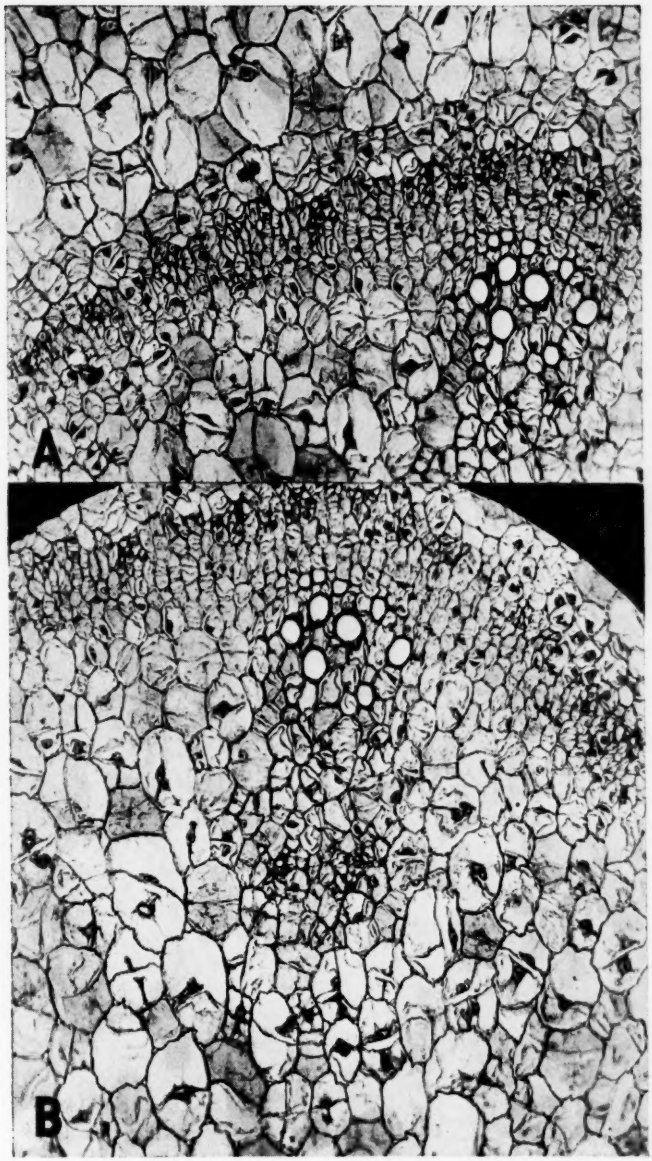

FIG. 6.—Same section as fig. 4 enlarged. *A*, details of outer region; *B*, of inner portions.

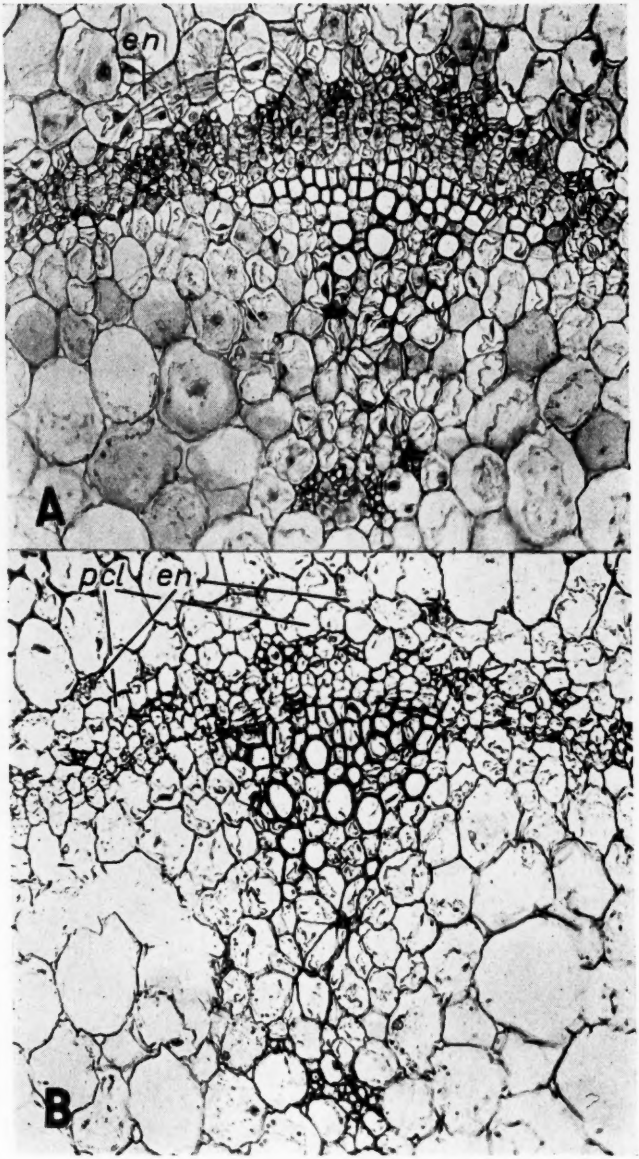

FIG. 7.—Sixty hours after treatment. *A*, approximately 1.5 mm. from cut surface. Meristematic activity confined to endodermis and external phloem. *B*, about 3 mm. below cut surface. Meristematic activity confined to cambium only. Other tissues approaching maturity.

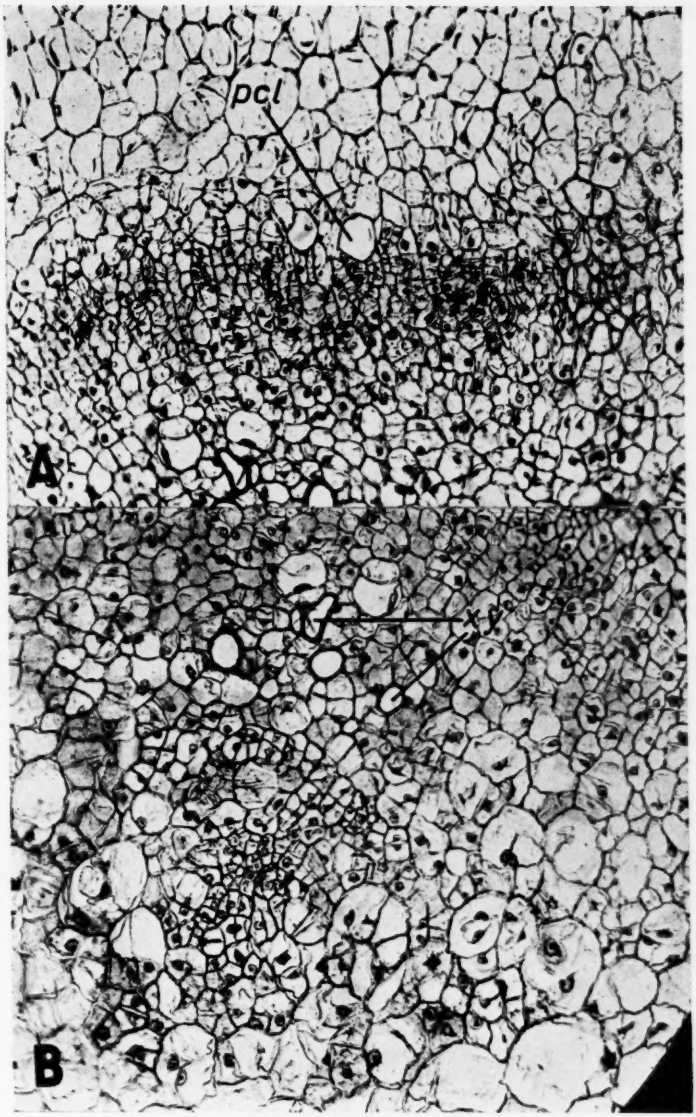

FIG. 8.—Eighty-four hours after treatment. *A*, approximately 200 μ below cut surface. Meristematic activity in cortical parenchyma and endodermis. Pericyclic cells more nearly mature. Outer phloem parenchyma highly meristematic. Ray parenchyma at right of center with many divisions. *B*, same section. Meristematic activity of xylem, internal phloem, and pith.

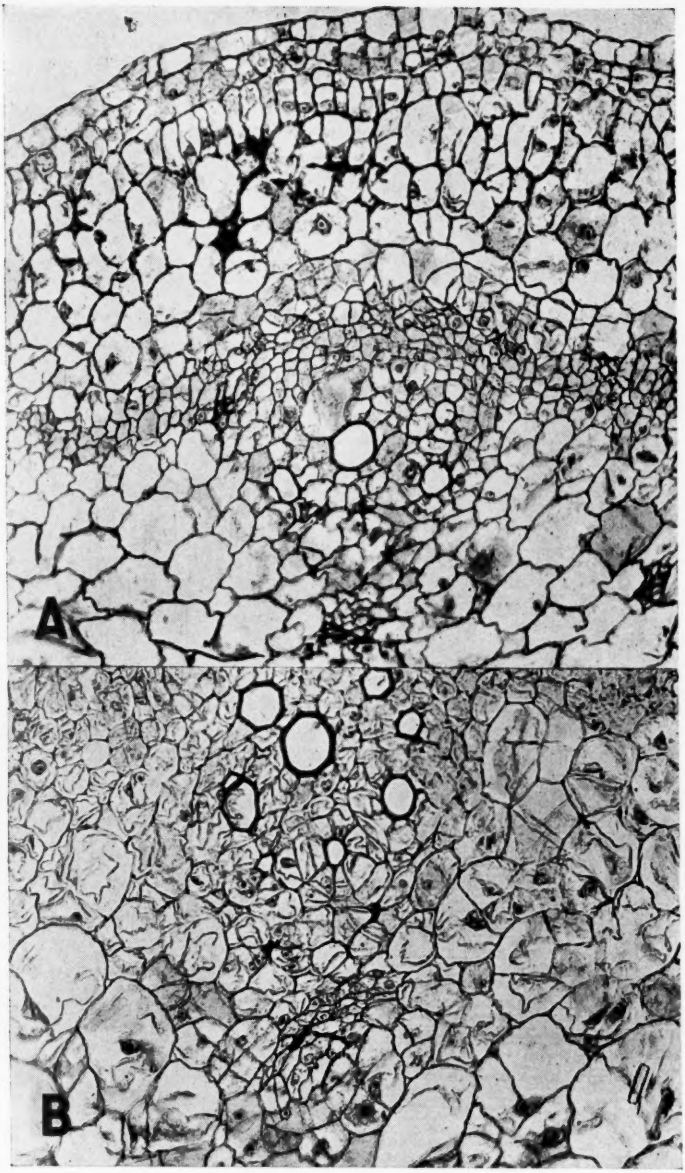

FIG. 9.—Ninety-six hours after treatment. *A*, section 200 μ below cut surface, above region of greatest activity. Endodermis meristematic. *B*, about 750 μ below cut surface, same bundle as in *A*. Great meristematic activity of all parenchymatous tissues.

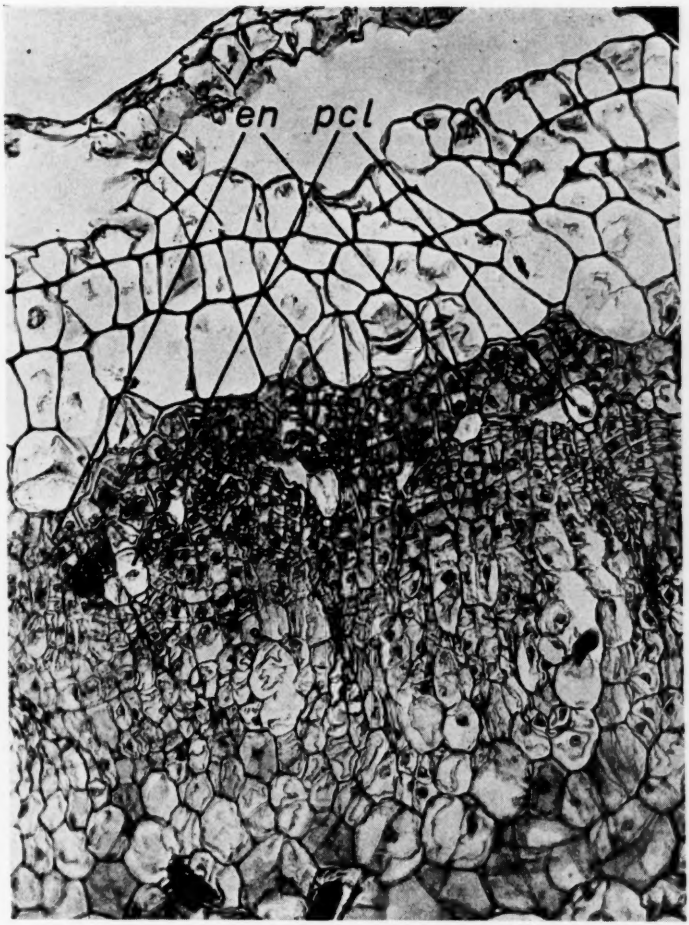

FIG. 10.—Ninety-six hours after treatment. Section through a ray at level slightly lower than fig. 8*B*. Endodermal derivatives in a band several cells wide. Some pericyclic cells nearing maturity, others crushed. Phloem parenchyma highly active.

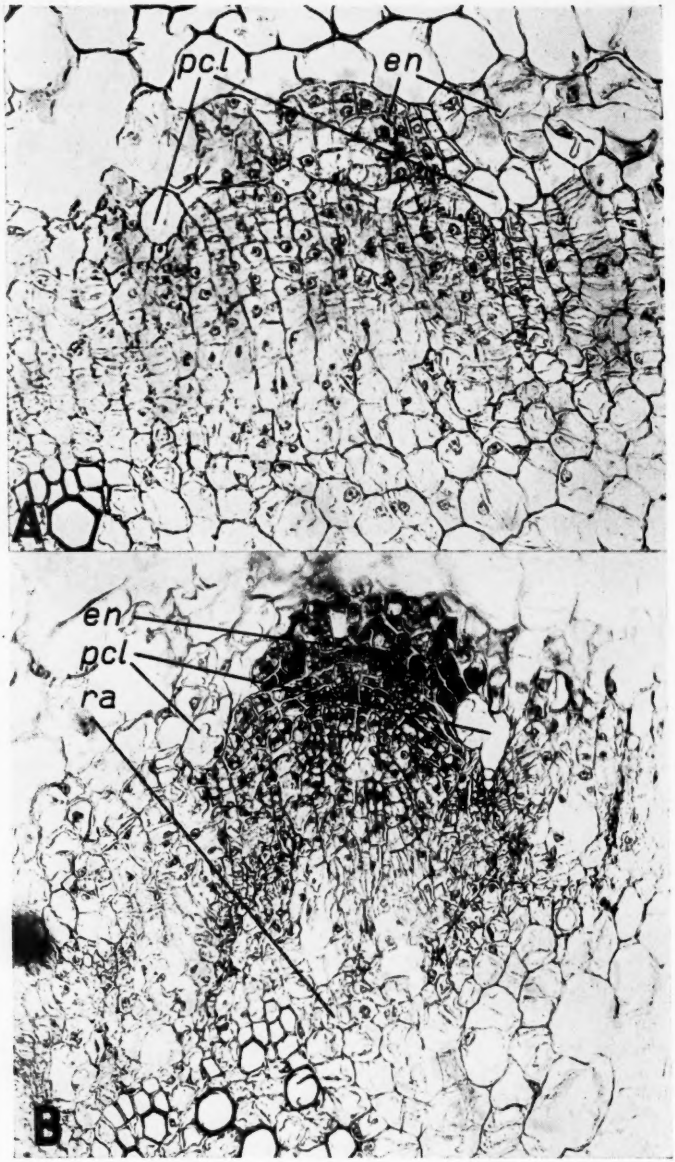

FIG. 11.—Ninety-six hours after treatment; 1.4 mm. below cut surface. *A*, endodermal derivatives constitute a band 3–5 cells wide. Pericyclic cells nearing maturity or crushed. Phloem parenchyma and ray cells highly meristematic. *B*, detailed view showing relation of adventitious roots and ray. The cap over primordium of adventitious root derived from proliferated endodermis.

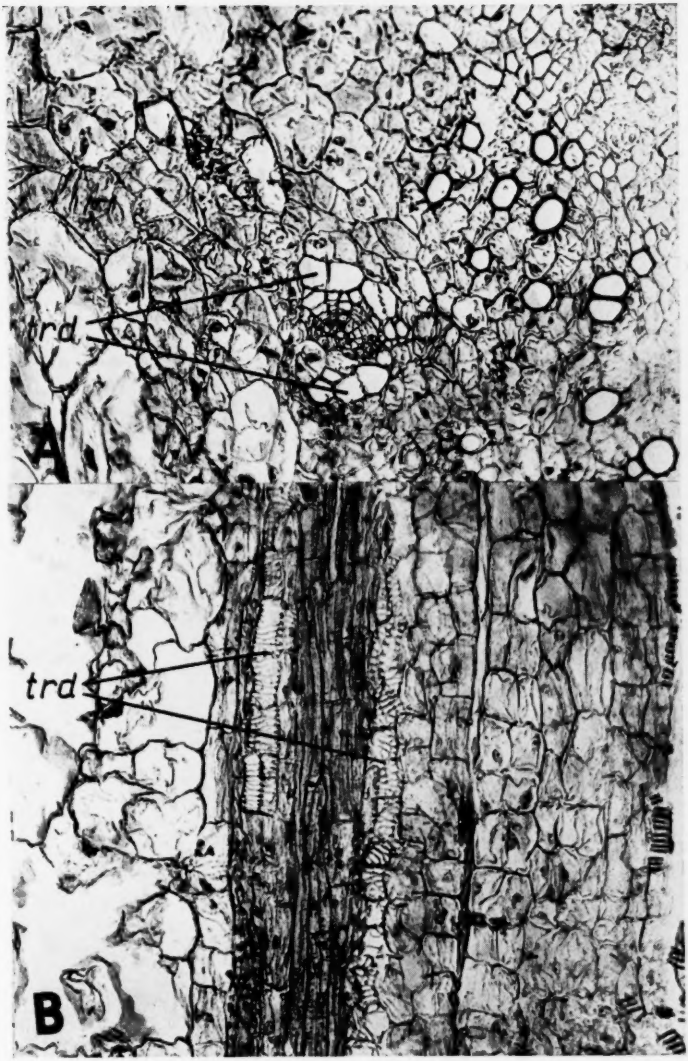

FIG. 12.—One hundred eight hours after treatment. Detail of region of internal phloem. *A*, cross section 1.2 mm. from cut surface. At center, group of sieve tubes and companion cells of internal phloem surrounded by tracheids derived from surrounding parenchymatous cells. *B*, longitudinal section. At left of center, strand of sieve tubes and companion cells with adjacent tracheids derived from surrounding parenchymatous cells as in *A*.

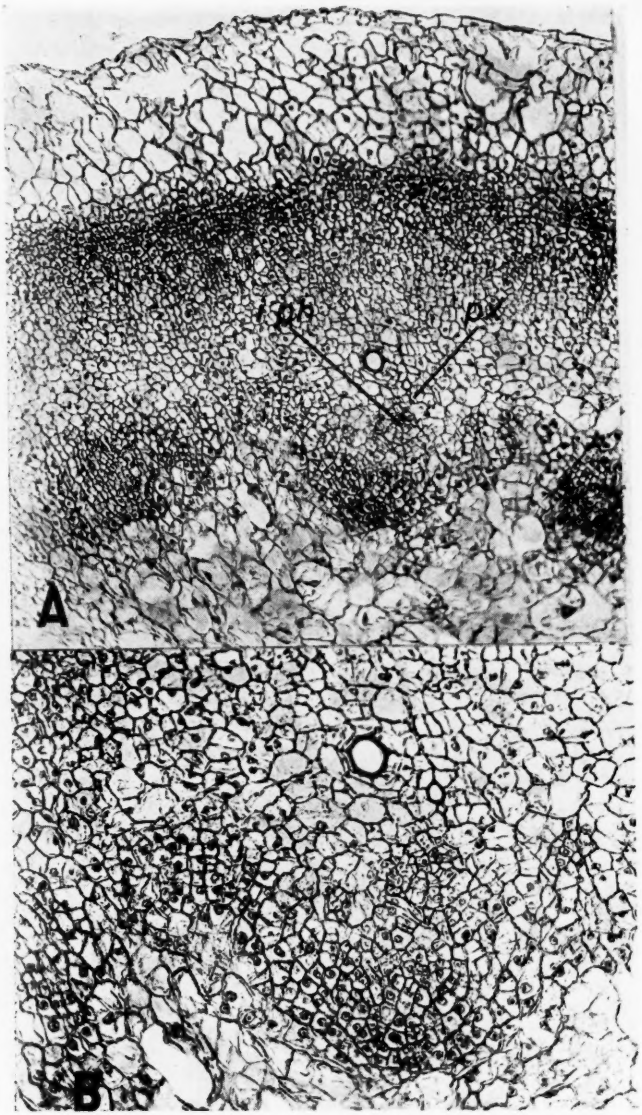

FIG. 13.—One hundred thirty-two hours after treatment; 400 μ below cut surface. *A*, all parenchymatous tissues centripetal to inner cortex actively meristematic. Beginnings of three roots in vicinity of internal phloem. *B*, enlarged central portion of *A*.

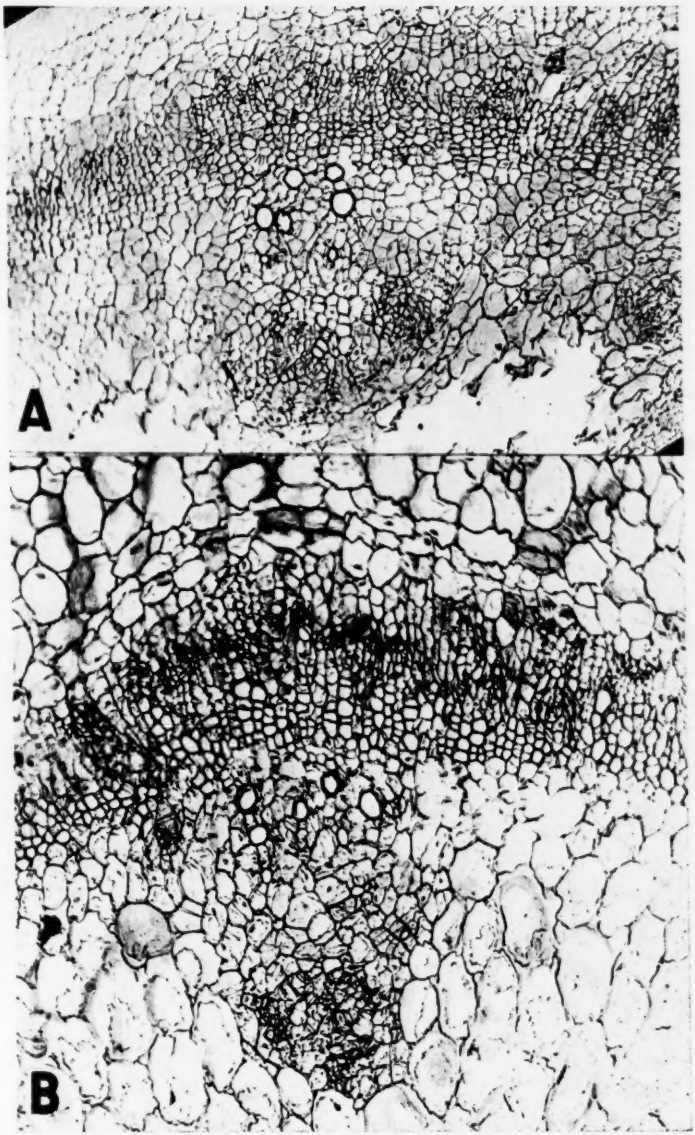

FIG. 14.—One hundred thirty-two hours after treatment. *A*, 700 μ from cut surface. Some of derived cells in region of internal phloem have matured as tracheids. Same stem as fig. 12. *B*, 1.8 mm. from cut surface. Same bundle as shown in *A*, slightly more enlarged. Greatest activity in region of pith adjacent to primary xylem and internal phloem. Fewer tracheids in region of internal phloem. Endodermis and external phloem also show proliferation.

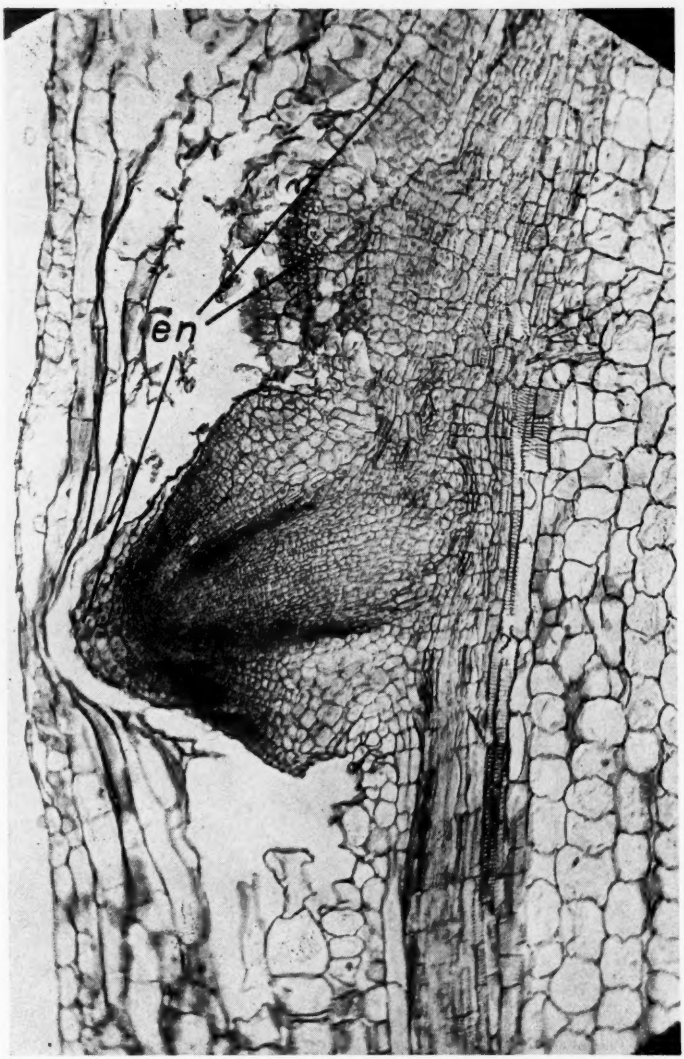

FIG. 15.—One hundred forty-four hours after treatment; median longitudinal section through adventitious root. Endodermis above root several cells in width. A portion of it has been broken away and caps the tip of root. Phloem also proliferated. There has been relatively little activity below the root.

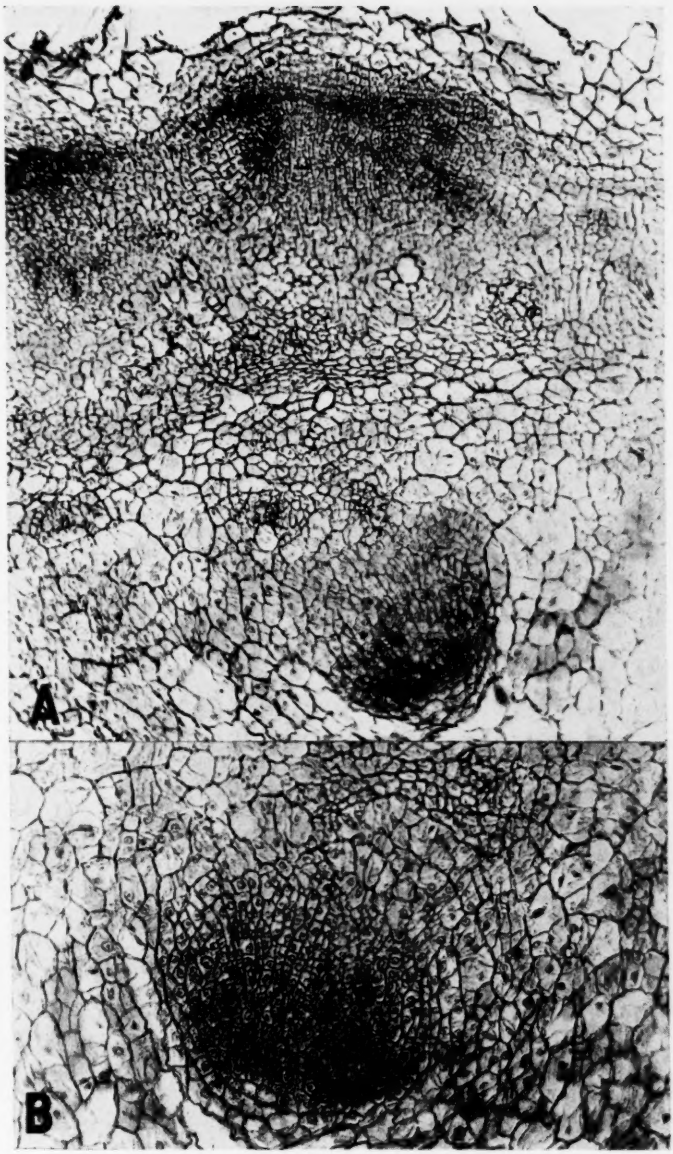

FIG. 16.—One hundred sixty-eight hours after treatment; 600 μ below cut surface. *A*, adventitious roots derived from vicinity of external and internal phloem. *B*, enlarged view of young internal root.

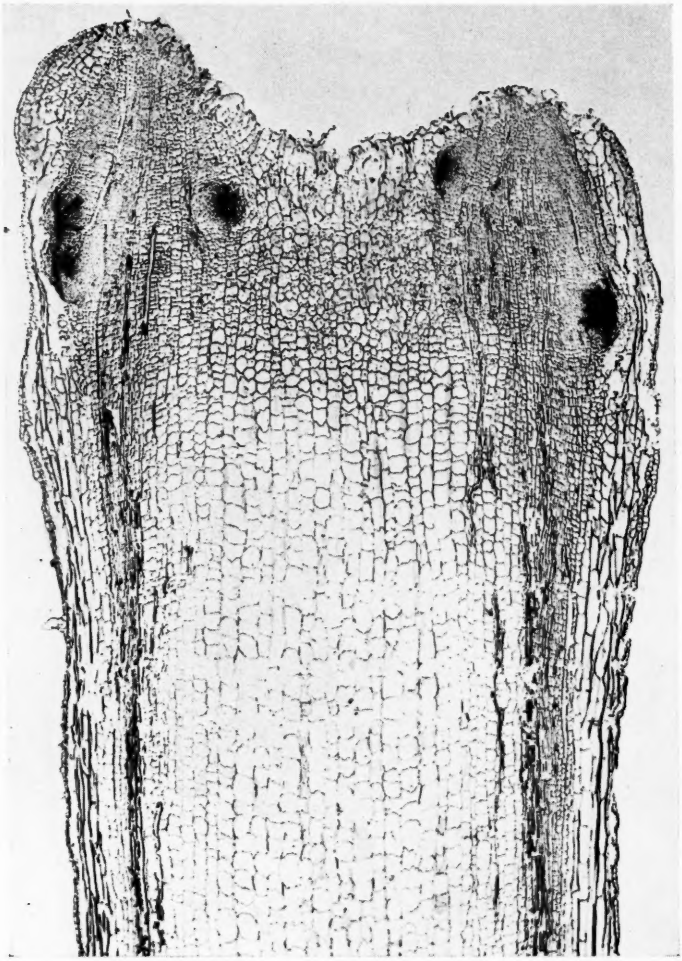

FIG. 17.—Two hundred sixteen hours after treatment; nearly median longitudinal section. Beginnings of both internal and external adventitious roots, above which there has been much greater proliferation of endodermis than below (*cf.* fig. 15). Pith has proliferated much more extensively several cells below treated surface than adjacent to it (*cf.* fig. 8).

FIG. 18.—One hundred ninety-two hours after treatment; transection through zone of internal and external adventitious roots. Pith is disintegrating. It has proliferated somewhat in this region but not so much as farther down the stem (*cf.* fig. 17).

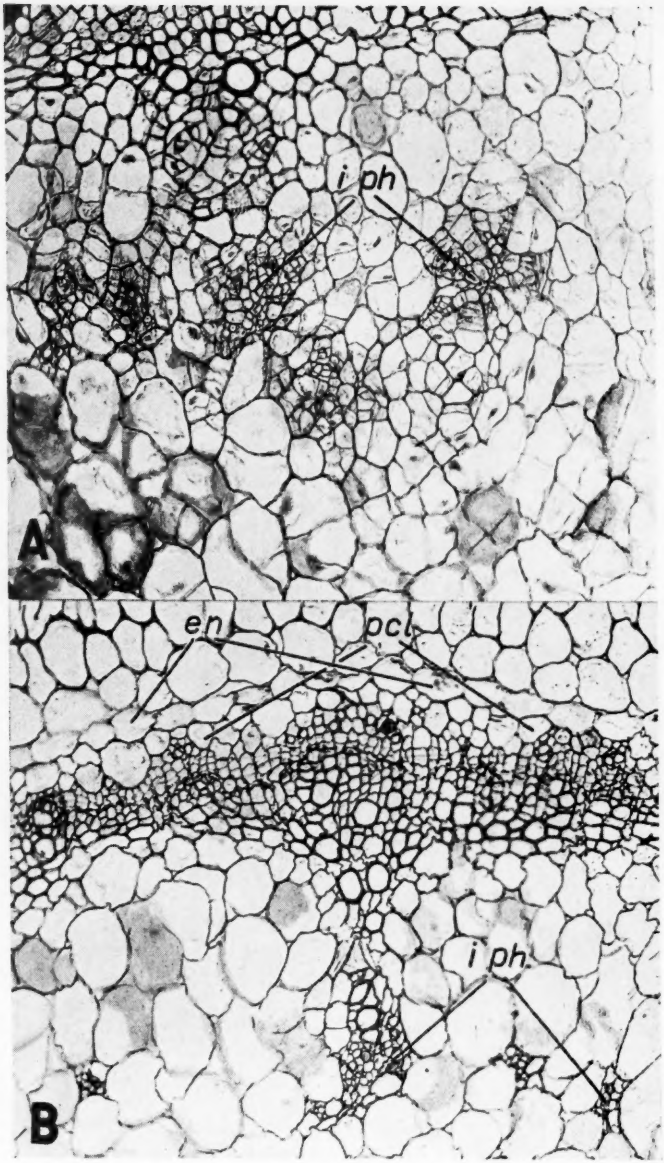

FIG. 19.—Two hundred sixteen hours after treatment. *A*, section 1.8 mm. below treated surface. Parenchymatous cells in vicinity of sieve tubes and companion cells of internal phloem highly meristematic. *B*, 4.0 mm. from treated surface, same stem. Cambium active but proliferation of other tissues slight. Some derivatives near internal phloem differentiated as tracheids.

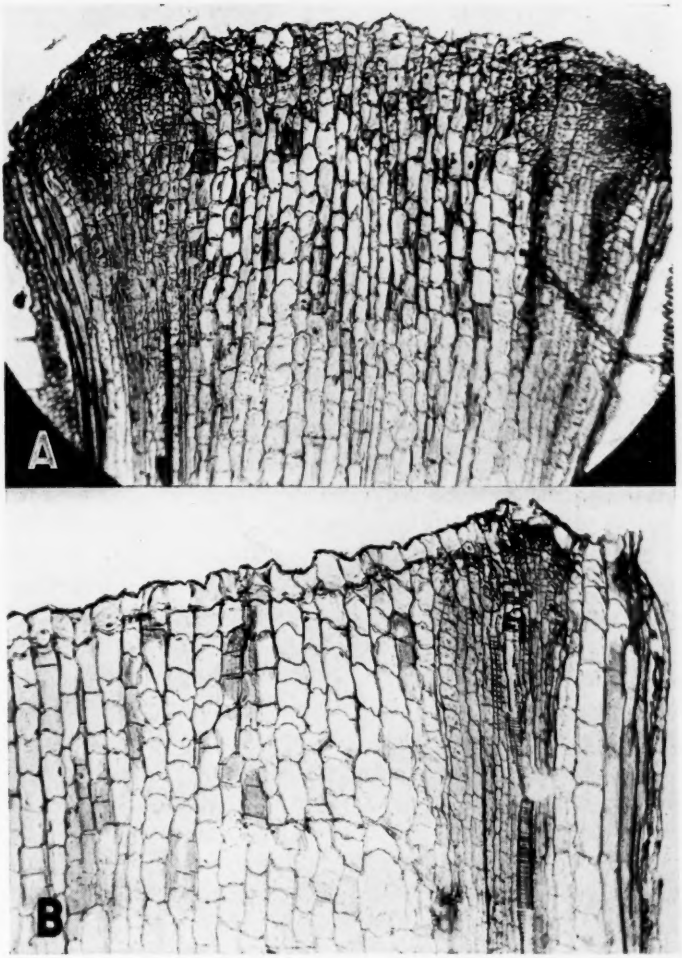

FIG. 20.—Twenty-three days after decapitation. *A*, cut surface untreated. Near it the phloem has proliferated, resulting in slight swelling of tip of stem. *B*, cut surface treated with lanolin only. A phellogen has developed across entire cut surface. Very little proliferation of other tissues except external phloem near the top.

STARCH.—The localization of starch in the treated stems was followed in detail during the experiment. Starch was concentrated in the endodermis and in the parenchymatous cells surrounding the xylem, and was abundant in the cortex and pith before any treatment was begun. The concentration of starch decreased following decapitation, regardless of the treatment. During the first 24 hours the stems that were treated with the indoleacetic acid mixture contained more starch than the controls which were treated with lanolin only. Following this period, the starch began to disappear in the cells surrounding the xylem in the treated stems. As time went on, in the plants treated with indoleacetic acid the starch began to decrease in the endodermis and in the pith cells which had become meristematic, but remained in those which were not dividing. As the tumors enlarged the concentration of starch decreased greatly in the proliferated endodermis. Immediately below the enlarged portion the starch was very abundant. The tumors were practically devoid of starch after 216 hours except for a layer three or four cells thick directly below the original cut surface. The cortical, endodermal, and pith cells in this layer retained their starch content throughout the experiment. Those treated with lanolin only contained abundant starch in the endodermis, cortex, and pith, except for a region three or four cells below the cut surface. Stems which were decapitated only were similar to the lanolin controls except that the starch was present in the cells up to the cut surface. Apparently the capacity of cells at the cut surface to digest starch was destroyed by the indoleacetic acid, since the lanolin controls contained no starch in a comparable area.

The endodermis adjacent to the young roots became completely void of starch. The root caps of all young roots contained abundant starch at first; this, however, soon disappeared.

Comparative responses of bean and tomato

The histological reactions of the tomato in response to indoleacetic acid are in many respects similar to those of the bean (1). In both the tissues of the cortex, phloem, rays, and pith become meristematic in response to treatment but the degree of the response differs. Moreover, the presence of an active internal phloem in tomato, in contrast

to the bean, results in further differences, which may be correlated with structure.

The degree of activity, tissue for tissue, is less in the tomato than in the bean, and the zone of activity does not extend so far down the stem. In the bean, vascular strands which resembled the tumor strands reported by SMITH, BROWN, and MCCULLOCH (2) were found in the endodermis and phloem. The only evidence of structures similar to these in the tomato was found arising from the internal phloem (fig. 12).

In the bean enormous tumors are produced, largely through long continued activity in the pith. Gross observations were made on several hundred tomato tumors over a period of two months, and under the conditions of these experiments little, if any, further growth was observed after the first ten days.

In the bean a ring of adventitious roots is formed as a result of activity in the phloem and rays. In the tomato two rings of roots are formed, the outer being similar in origin and development to that of the bean. The roots of the inner ring arise centripetal to and in close association with the internal phloem, and grow into the pith.

Many studies have shown that treatment with indoleacetic acid results in the formation of adventitious roots, in many cases on stems that would not ordinarily form such roots. The investigations on bean and tomato have shown that, in these two plants at least, treatment will induce the formation of such roots in tissues that ordinarily do not produce them. In both plants treatment with indoleacetic acid has resulted in the formation of adventitious roots from derivatives of the phloem or rays.

In the tomato adventitious roots are formed also from derivatives of the internal phloem and pith. The fact that indoleacetic acid induces formation of adventitious roots in tissues other than the pericycle indicates that its action is not merely a speeding up of the normal process of adventitious root formation. The roots formed in the external phloem, under favorable conditions, grow out through the cortex and have the appearance of adventitious roots formed on untreated plants. So far the roots formed from the internal phloem and pith have been observed to grow into the pith a short distance and then cease further extension. Whether these roots could be in-

duced to continue their growth by some type of treatment of the stem is not yet known. Structurally they have the appearance of roots formed anywhere else in the plant. This indicates that the treatment with indoleacetic acid has not fundamentally altered the hereditary make-up of the individual cells of the plant. It is obvious that the usual course of development of some of the tissues is altered, but no types of cells or types of tissues not customarily formed were differentiated. Such differences as exist are those of degree, rather than of kind. Generalization as to causes in relation to the developmental patterns resulting from treatment with indoleacetic acid must await further cytological and histological investigation.

Summary

1. Tomato seedlings were decapitated above the second lobed leaf and a mixture of 20 mg. of indoleacetic acid in 1 gm. of lanolin was applied to the cut surface. Similarly, decapitated plants were kept as controls, either untreated or with pure lanolin applied to the cut surface.

2. Gross observations and histological and microchemical studies were made of treated and control material collected at frequent regular intervals for a period of 216 hours after treatment.

3. The internode terminated by the cut surface fails to enlarge after decapitation unless the cut surface is treated with indoleacetic acid-lanolin mixture. The internodes so treated enlarge at about the same rate as do the internodes below.

4. The first change observed after treatment was enlargement of cells of the epidermis and cortex.

5. Many of the tissues of the stem became meristematic in response to the treatment, although most of this activity was confined to a zone 0.5 to 2 mm. from the treated surface. Most parenchymatous cells in this zone underwent some divisions; the endodermal cells, external and internal phloem parenchyma, and pith cells adjacent to the internal phloem exhibited the greatest activity. There were, however, certain tissues comprising a small portion of the stem whose living cells showed little or no meristematic activity. These include epidermis, most of the pericycle, sieve tubes and companion cells, and internal fibers.

6. A ring of adventitious roots was formed around the stem as a result of activity in the external phloem. Portions of the active band of endodermal cells covered the tips of these roots as they pushed through the cortex. Another ring of adventitious roots was formed near the pith as a result of activity of cells of the internal phloem and adjacent pith.

7. Nitrates were more concentrated in the controls than in the stems treated with indoleacetic acid. The concentration of protein in the treated stems increased in those areas where cell divisions became most abundant. Starch disappeared from the cells of the treated stems as the tumors enlarged, but remained in the controls.

U.S. Horticultural Field Station
Beltsville, Maryland

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EFFECT OF EXTRACTS FROM THE CORN PLANT ON GROWTH OF EXCISED ROOT TIPS[1]

WILLIAM J. ROBBINS AND VIRGINIA B. WHITE

(WITH THREE FIGURES)

Introduction

In the experiments performed in this laboratory on the cultivation of excised corn root tips under sterile conditions, extracts of corn grains and other parts of the corn plant have occasionally been used to supplement the mineral solution and dextrose used as a nutrient medium. The corn grain is the natural source from which a successful medium for the growth of excised roots should be derived, since the root attached to the grain may secure from that source everything it requires for early growth, save water and oxygen. The earlier experiments, however, indicated that extracts from the grain or from the corn top were of little benefit or were injurious (4). In the present paper results are reported on the effect (1) of milk from young grains, (2) of the diffusate into agar from germinated grains, (3) of the diffusate into agar from root tips and shoot tips, (4) of root extracts, (5) of extracts of germinated grains, and (6) of experiments in which the solution containing various supplements was renewed during the growth of the roots.

MATERIALS AND METHODS.—The materials used and methods employed have been described in an earlier paper (7) and will not be repeated here.

Experimental results

MILK FROM YOUNG CORN GRAINS.—In the few experiments performed, milk from corn in the roasting ear or soft milk stage was toxic at the higher concentrations and ineffective at lower concentrations, at least in an agar medium.

In one experiment, roots originally 2 mm. long were grown in 125 cc. Erlenmeyer flasks on qualitative filter paper moistened with unheated milk from corn grains. In one series the undiluted milk was

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used; in the other, milk diluted ten times with redistilled water. Because of the difficulty in securing the milk in a sterile condition, the series were not large. However, the milk at the concentrations used was decidedly injurious to the excised root tips.

In a second experiment the milk was added at different concentrations to solution C[2] plus 0.5 per cent agar and sterilized. At a concentration equivalent to the milk from 1.5 or 3 grains per flask the milk was toxic; at concentrations from 0.5 grain or less no effect on the growth of the roots was noticed.

DIFFUSATE FROM GERMINATED CORN GRAINS.—The diffusate from a germinated grain was beneficial to the growth of root tips less than 1 mm. in length in solution CF plus agar.

The experiments were carried out as follows. Corn was germinated under sterile conditions, and when the seedling root was about 7 cm. long both root and shoot were removed. The grain was cut longitudinally in half at right angles to the flat surface, and each half was transferred to a sterile petri dish containing a layer of agar. The half grain was placed in the center of the dish and the cut surface pressed firmly down against the agar. By testing the agar at different distances from the grain with Fehling's solution, the xanthoproteic reaction, and Millon's reagent, it was found that reducing sugar diffused from the grain to the edge of the dish in three days; the amino acids, in six days. This was taken to indicate that whatever diffuses from the grain would probably be generally distributed through the agar in a petri dish within seven days after the split grain was placed therein.

Plates of CF agar were prepared and in some of them a half grain was placed. After seven days excised root tips were placed in the plates, which were incubated at room temperature in the dark. In some cases the half grain was removed before the roots were placed in the dishes; in other cases the grain was left in the dish. The growth of the roots was measured daily and the experiments were limited to ten days or two weeks because of the drying out of the agar in the

[2] Solution C contained calcium nitrate, 50 p.p.m.; magnesium sulphate, 10 p.p.m.; potassium dihydrogen phosphate, 10 p.p.m.; ferric chloride, 1 p.p.m.; dextrose, 2%. Solution CF consisted of solution C plus manganese chloride, 0.1 p.p.m.; zinc chloride, 0.1 p.p.m.; and sodium borate, 0.1 p.p.m.

dishes. Under such conditions a beneficial effect of the grain was found for roots less than 1 mm. in length; longer roots were not benefited although different results might have been found had conditions permitted the experiments to run for longer periods. A typical experiment is summarized in table 1.

DIFFUSATE FROM ROOT TIPS OR COLEOPTILE TIPS.—WENT (9) secured the growth hormone from coleoptile tips by permitting it to diffuse into agar. The effect of the diffusate from coleoptile tips and root tips on the growth of excised corn root tips was determined by

TABLE 1

EFFECT OF DIFFUSATE FROM ONE-HALF CORN GRAIN ON GROWTH OF ROOTS LESS THAN 1 MM. IN LENGTH. GROWN 10 DAYS IN PETRI DISHES AT ROOM TEMPERATURE IN DARK

MEDIUM	NO. ROOTS	AVERAGE ORIGINAL LENGTH (MM.)	AVERAGE FINAL LENGTH (MM.)
CF agar.....	14	0.60	6.07
CF agar plus half grain	18	0.69	12.56

the writers. The results were not definite although some beneficial effect was secured from the root tips and some detrimental effects from the coleoptile tips. The experiment was performed as follows.

Petri dishes containing solution CF plus 0.5 per cent agar were prepared and divided into three groups. In those of one group root tips 2 mm. long were placed on end with the cut surface pressed down into the agar. In a second group 2 mm. coleoptile tips were similarly placed. The third group was undisturbed. After four days the root tips and coleoptile tips were removed and on the spots formerly occupied by them excised root tips of less than 1 mm. original length were placed. Measurements of growth were made daily, the experiment being terminated after seven days. The curves giving the growth in mm. per day (fig. 1) and the final lengths (table 2) show that the growth in the plates with diffusate from the root tips was somewhat greater than the check, and that in the plates with diffusate from the coleoptile tips it was somewhat less than the check.

EXTRACTS FROM ROOTS.—It was thought that the roots themselves might contain some material which would be beneficial to the growth of excised root tips. Extracts of different portions of the terminal 15 mm. of the primary roots of young seedlings were used at various concentrations. In solution C plus agar the root extract was beneficial when added at a concentration of 0.1 ,2.5, 3, 5, and 10 roots per flask containing 30 cc. of medium in which 1 or 2 mm. root

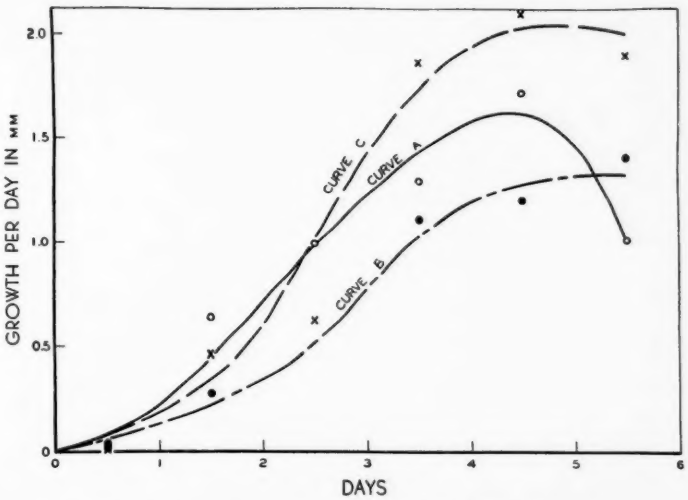

FIG. 1.—Growth in mm. per day of short root tips in solution C plus agar (curve *A*), solution C plus agar on which a coleoptile tip had been placed (curve *B*), and in solution C plus agar on which a root tip had been placed (curve *C*).

tips were grown individually. The benefit increased up to and including the extract of 5 roots; the effect of the extract from 10 roots was less marked. The extracts were made from 3 mm. portions of the roots. In some cases the extracts from the more basal portions of the tip were somewhat more beneficial than from those which included the tip itself.

The extracts were prepared by cutting the terminal 15 mm. of the primary roots of young seedlings into five 3 mm. portions. The segregated parts of the roots were heated to boiling in redistilled water

and macerated. The extract was filtered and filtrate added to the medium. Although qualitative filter paper was used in filtration, the amount of filter paper extract added per flask was too small to be significant. In table 3 the results of a single experiment with root tips 2 mm. in length are given.

TABLE 2

EFFECT OF DIFFUSATE FROM 2 MM. ROOT TIPS OR 2 MM. COLEOPTILE TIPS ON GROWTH OF SHORT EXCISED ROOT TIPS IN SOLUTION CF PLUS AGAR. PERIOD OF GROWTH 7 DAYS

TREATMENT	NO. ROOTS	LENGTH			
		AVERAGE ORIGINAL (MM.)	AVERAGE FINAL (MM.)	MAXIMUM (MM.)	MINIMUM (MM.)
None	17	0.59	6.3	14.0	1.5
Root tips	21	0.62	7.6	22.0	1.0
Coleoptile tips	17	0.66	5.1	15.5	1.4

TABLE 3

EFFECT OF EXTRACT OF 3 MM. PORTIONS OF SEEDLING CORN ROOTS ON GROWTH OF EXCISED CORN ROOT TIPS. GROWN IN 30 CC. OF SOLUTION C PLUS 0.5 PER CENT AGAR IN 125 CC. ERLENMEYER FLASKS FOR 46 DAYS AT ROOM TEMPERATURE IN DARK. ORIGINAL LENGTH 2 MM.

ADDITION TO SOLUTION C PLUS AGAR	NO. ROOTS	AVERAGE FINAL LENGTH (CM.)	AVERAGE NO. SECONDARY ROOTS	MAXIMUM LENGTH (CM.)	MAXIMUM NO. SECONDARY ROOTS	MINIMUM LENGTH (CM.)	MINIMUM NO. SECONDARY ROOTS
None	11	17.0	66	44.4	116	4.1	10
Extract of 5 roots	9	28.5	99	56.0	189	8.5	28
Extract of 10 roots	11	23.0	52	67.4	153	4.1	7

EXTRACTS FROM CORN GRAINS.—Extracts of germinated corn grains were found to be injurious at higher concentrations and beneficial at lower concentrations.

The extracts were prepared from grains which had been germinated in dishes between sheets of filter paper. When the roots of the seedlings were from 10 to 15 cm. long, the shoots and roots were removed and the grains ground in a mortar. The ground material was

covered with about three times its volume of redistilled water and after 24 hours at room temperature the liquid was filtered through several layers of washed cheesecloth. The filtrate was used in the experiments.

When extract equivalent to that from one grain or two grains was added to 30 cc. of 2 per cent glucose or of solution C, distinct injury was produced. Root tips originally 1 cm. long made little growth, the roots swelled, the cortex wrinkled in ridges perpendicular to the long axis of the root and in some cases split away from the central cylinder. The apical meristem did not swell materially. A microscopic examination of some of the roots after 30 days in the solutions showed the cortical cells very much enlarged, some of them 0.3 mm. in diameter and many of them still alive. Enlargement had occurred to within 1 mm. of the root tips. Whether the apical meristem was still alive could not be determined by microscopic examination. Some of the cultures containing the grain extract were diluted with sterile solution C at the end of four weeks and in about one week new growth appeared at the root apex. The new tip was slender and grew out through the tissues of the old tip, representing probably the regeneration of a new tip from the pericycle. The maximum new growth made was 1 cm.

While not encouraging from the standpoint of the cultivation of excised root tips, these results are of interest because the injury is similar in character to that found in abnormal roots grown in solution C and because of the hypertrophy of the cortical cells, which remained alive for four weeks.

When used at a concentration equivalent to the extract of 0.1 of a grain per 30 cc. of medium in which an individual root was grown, beneficial effects were found as illustrated by the following experiment:

Roots originally 1, 2, 3, 4, or 5 mm. long were grown individually in 30 cc. of solution C plus 0.5 per cent agar or the same medium plus grain extract in 125 cc. Erlenmeyer flasks, for 70 days at room temperature in the dark. The growth was measured at intervals during the first 20 days. The results are summarized in table 4.

The total growth in solution C plus agar was approximately proportional to the length of the original tip. This is shown in table 5

where the growth per mm. of original length is given. These results are contrary to those secured in the modified Pfeffer's solution and previously reported (4) where root tips varying from an average original length of 0.35 to 6.06 cm. reached approximately the same average final length. Apparently the growth in the latter solution was limited by the medium. Since the average growth in solution C plus agar was proportional to the original length of the root tip, it

TABLE 4

EFFECT OF GRAIN EXTRACT ON GROWTH OF EXCISED CORN ROOT TIPS ORIGINALLY 1, 2, 3, 4, OR 5 MM. LONG GROWN 70 DAYS IN 30 CC. OF SOLUTION C PLUS 0.5 PER CENT AGAR IN 125 CC. ERLENMEYER FLASKS AT ROOM TEMPERATURE IN DARK

ADDITION	ORIGINAL LENGTH (MM.)	NO. ROOTS	AVERAGE FINAL LENGTH (CM.)	AVERAGE NO. SECONDARY ROOTS	MAXIMUM LENGTH (CM.)	MAXIMUM NO. SECONDARY ROOTS	MINIMUM LENGTH (CM.)	MINIMUM NO. SECONDARY ROOTS
None	1	11	5.3	21	24.5	106	1.5	1
Grain extract	1	12	11.6	45	41.5	206	1.3	0
None	2	14	14.4	65	47.1	220	4.2	12
Grain extract	2	11	17.9	59	37.9	122	3.2	10
None	3	12	19.1	84	36.8	163	8.9	44
Grain extract	3	14	22.3	81	53.8	206	6.6	18
None	4	11	27.0	128	58.1	236	9.3	42
Grain extract	4	15	25.5	98	77.4	296	9.3	25
None	5	9	29.6	124	51.0	220	17.0	74
Grain extract	5	14	29.5	245	68.0	238	17.0	73

would seem that the growth in that medium was limited by materials originally contained in the root tip. The grain extract supplemented these materials and permitted greater growth of root tips originally 1, 2, or 3 mm. long to occur. The grain extract did not affect the growth of tips of longer original length, probably because the experiments were not continued long enough to exhaust the larger amount of material contained in the longer root tips.

The effect of the grain extract in relation to the original length of the root tips may be calculated in the following way. Assuming that the average growth per mm. of length of the original root tips in the agar medium without grain extract is 63 mm. (table 5), then the effect of the grain extract on the 1 mm. root tips was to produce 116

minus 63 or 53 mm. additional growth. For the 2 mm. root tips the effect of the grain extract similarly calculated was 179 minus 126 or 53 mm.; for the 3 mm. root tips, 34 mm.; for the 4 mm. root tips, 3 mm.; and for the 5 mm. root tips, −20 mm.

No evidence can be deduced from these experiments as to whether the deficiency in the modified Pfeffer's solution supplied by the agar medium or the deficiency in the root supplied by the grain extract is organic or inorganic, qualitative or quantitative.

TABLE 5

GROWTH PER MM. OF ORIGINAL LENGTH OF ROOT TIPS GROWN IN 30 CC. OF SOLUTION C PLUS 0.5 AGAR OR IN SAME MEDIUM PLUS GRAIN EXTRACT (SEE TABLE 4)

ORIGINAL LENGTH (MM.)	GROWTH PER MM. ORIGINAL LENGTH	
	CHECK (MM.)	GRAIN EXTRACT (MM.)
1	53	116
2	72	89
3	64	74
4	67	64
5	59	59
Average	63	80

Further evidence on the effect of extracts on the growth of excised roots was secured by another procedure.

EFFECT OF ADDITIONS TO SOLUTION C AND OF CHANGE OF SOLUTION.—In a preliminary experiment root tips of 1 cm. original length were grown in 125 cc. Erlenmeyer flasks containing 40 cc. of 2 per cent dextrose and the extract of 0.1 or 0.01 of a corn grain. By marking the position of the tip of the main root on the outside of the flask by means of a wax pencil, the daily growth of individual roots was recorded at intervals. When the growth of a particular root decreased to 0.5 cm. or less per day, the solution covering it was replaced by fresh solution. Some roots showed no response to this treatment, others increased their rate of growth after the replace-

ment of the solution, and after passing through a maximum, again showed a decline. In some cases a second replacement caused a new burst of growth. In fact one root after a second supply of fresh solution showed a growth of 2.2 cm. per 24 hours between the fifty-seventh and sixtieth day of cultivation, when the experiment was discontinued. These results were interpreted to mean that some material in the grain extract was exhausted by the growth of the root and supplied in the fresh solution. Staling products were not thought important because in some cases similar results were secured when the extract was added to the old solutions.

In a more elaborate experiment, excised corn root tips originally 2 mm. long were grown in 30 cc. of solution in 500 cc. Erlenmeyer flasks at room temperature in the dark. The following media were used: solution C, solution C plus the extract of 0.1 grain per flask, solution C plus 666 p.p.m. of autolyzed yeast, and solution C plus root extract equivalent to five 3 mm. portions per flask. The original root tips were pretreated on agar, and whenever the tips became translucent during their growth the flask was tilted and the root exposed to the air until the translucent condition disappeared, or until danger of injury from desiccation made it advisable again to submerge the tip. By marking the position of the end of the root on the outside of the flask at intervals the rate of growth of each root was noted, and when the rate decreased to 0.5 cm. or less per day the old solution was replaced by fresh. This procedure was continued until the root ceased growth.

A summary of the results of this experiment given in table 6 shows that each of the three extracts was beneficial to the roots. Under the conditions of this experiment the autolyzed yeast was most beneficial, the root extract least beneficial.

The response to the change of medium was very slight in solution C. Only four roots out of fourteen could be interpreted as being favorably affected by a change of solution C, and of these only one showed clear evidence of an increase in the rate of growth, and the effect in this case was not great.

A change in the medium was frequently followed by a marked increase in the rate of growth when solution C plus autolyzed yeast, grain extract, or root extract was used. All of the roots (17) in the

yeast series responded by increased growth rates to the first change of solution, ten of fifteen in the grain extract series, and five of twelve in the root extract series. The number of changes to which a response was made varied with the individual root; some responding to one change only, others to several. The difference between solutions and between individual roots is shown by the average number of days the roots grew in each solution; for solution C it was 13 days (range from 5 to 26); in the root extract, 20 (range from 9 to 36); in the grain extract, 29 (range from 11 to 56); in autolyzed yeast extract, 36 (range from 17 to 100).

TABLE 6

GROWTH OF 2 MM. EXCISED CORN ROOT TIPS IN SOLUTION C AND SAME SOLUTION PLUS VARIOUS SUPPLEMENTS; OLD SOLUTIONS REPLACED BY FRESH WHENEVER RATE OF GROWTH SHOWED MARKED DECLINE. GROWN AT ROOM TEMPERATURE IN DARK

Addition to solution C	No. roots	Average final length (cm.)	Average no. secondary roots	Maximum length (cm.)	Maximum no. secondary roots	Minimum length (cm.)	Minimum no. secondary roots
None	14	9.4	49	22.5	169	2.7	0
Grain extract	15	21.2	82	82.0	271	5.8	18
Autolyzed yeast	19	41.8	163	145.0	285	8.2	27
Root extract	13	19.3	88	53.0	179	5.0	20

The best single example of the beneficial effect of changing the medium was a root which grew in solution C plus autolyzed yeast for 100 days, reached a final length of 145 cm., and produced 285 secondary roots. Its final appearance is shown in figure 2. The rate of growth in mm. per day is shown in figure 3. The solution on this root was changed at the end of 42, 66, 85, and 91 days. In the first period the rate of growth attained a maximum and then declined. The drop in the curve at 11 to 15 days represents a lag caused by the partial drying of the root tip which resulted from tilting the flask in an attempt to correct a slightly abnormal condition of the tip. Replacement of the solution caused a new burst of growth which was repeated in the third, fourth, and fifth periods. As may be noted in figure 3, however, the length of the periods in which growth equaled

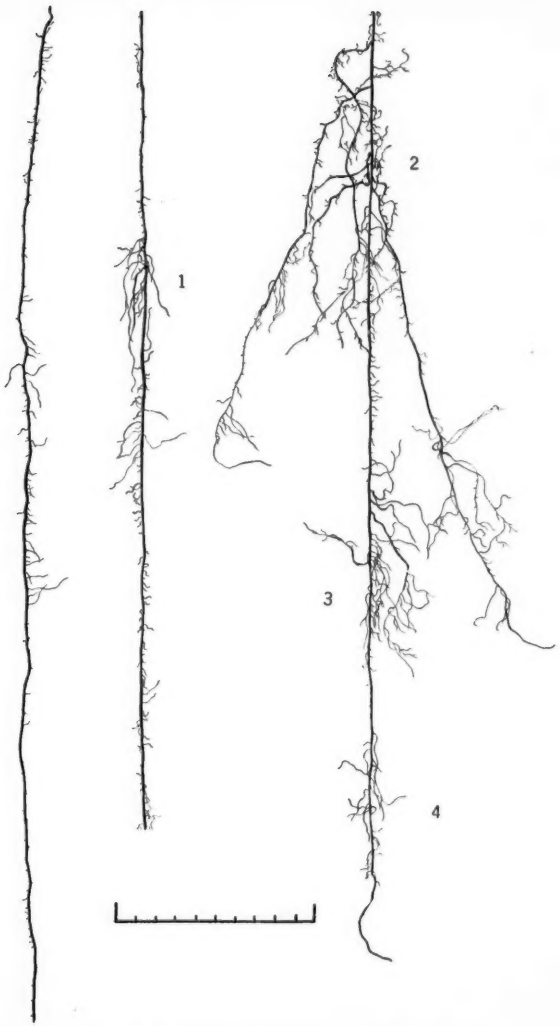

FIG. 2.—Root grown in solution C plus 666 p.p.m. of autolyzed yeast, old solutions being replaced with fresh when rate of growth showed considerable decline. Entire root is shown in the three pieces. Note groups of long branch roots at 1, 2, 3, and 4, which are located at positions corresponding to length of root when solution was changed. Original length 2 mm.; final length 1450 mm. Period of growth 100 days. Scale 10 cm.

or exceeded 0.5 cm. per day decreased with each change of the solution, and the maximum rate of growth was also less. In other words, changing the solution became less effective as time went on.

Groups of long branch roots were produced at positions corresponding to the length of the root when the solution was changed. Whether this was a response to the slower growth of the main root tip previous to the change or to the fresh solution is uncertain. We are inclined to think it was due to the latter.

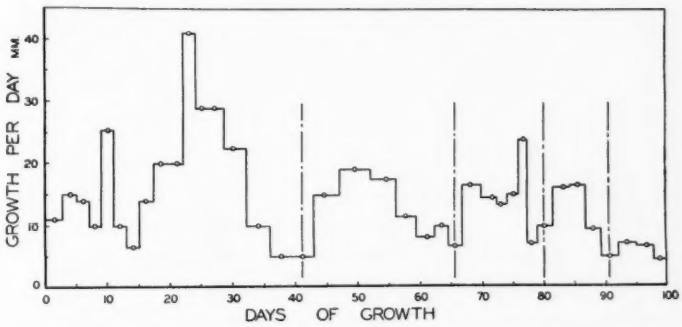

FIG. 3.—Rate of growth in mm. per day of root in solution C plus autolyzed yeast. Broken vertical lines show when old solution was replaced by fresh (see fig. 2).

Discussion

The problem with which we are primarily concerned may be stated briefly as follows: What are the conditions necessary for the continued growth of excised corn root tips? The media used, composed of solutions of inorganic salts and dextrose, have not proved satisfactory, either because they are deficient qualitatively or quantitatively in one or more nutritional requirements, or because they are injurious on account of a lack of balance, the presence of some specific toxic constituent, or some other condition. While recognizing the difficulty of separating these causes and the possibility that our results may be due to one or to several, we have been inclined to interpret them as due to one or more deficiencies in the mineral nutrient solutions containing dextrose which we have used.

On this basis, the beneficial effects on the growth of excised corn root tips reported in this paper or earlier (**3**, **7**) from the addition to such a medium as solution C of agar, water extracts of agar, qualitative filter paper, water extracts of qualitative filter paper, autolyzed yeast, peptone, corn grain extracts, and corn root extracts would be due to the correction in whole or in part of nutrient deficiencies in that solution. It is possible that a medium, such as solution C, is deficient for the growth of excised corn root tips in two respects; one inorganic in character, which is supplied by the salts contained in agar (or in qualitative filter paper) and the other, perhaps organic, which is supplied in whole or in part by autolyzed yeast, corn grain extracts, and similar supplements. The evidence at hand indicates that the addition of corn grain extract, corn root extract, or autolyzed yeast to solution C or to a modified Pfeffer's solution does not make those media entirely satisfactory for the growth of excised corn root tips. At the same time the beneficial effects of the addition of corn grain extract to solution C plus agar suggests that the latter medium is not entirely adequate.

It is possible that an inorganic medium plus dextrose may be found which is sufficient for the continued growth of excised corn root tips. At present we are inclined to believe that organic material in addition to dextrose is necessary, perhaps organic nitrogen. As a nutrient medium, one containing extracts of agar or qualitative filter paper and dried brewers' yeast, corn grain extract, or peptone in addition to dextrose and to the salts in solution C would seem most promising.

SÖHNGEN (**8**) found the addition of filter paper to Beijerinck's solution to improve the growth of *Azotobacter* and to increase its nitrogen fixation as much as five times. This was especially true when the filter paper extended above the medium. He explains the results on the basis of adsorption and better aeration.

ALLYN and BALDWIN (**1**) found the addition of powdered agar, yeast water, and ground filter paper beneficial to the growth of *Rh. trifolii* in certain mannitol media. They are inclined to explain the beneficial effects of these supplements as due to their action on the oxidation-reduction potential of the medium.

We would explain the beneficial action upon the growth of excised corn root tips of the various supplements we have used on the basis of nutrient deficiencies.

Summary

1. Milk from immature corn grains was injurious or without benefit to the growth of excised corn root tips.

2. The diffusate from germinated corn grains benefited the growth of excised corn root tips in an agar medium containing mineral salts and dextrose.

3. The diffusate into agar from coleoptile tips slightly inhibited the growth of excised root tips less than 1 mm. long; that from root tips slightly favored the growth.

4. Water extracts of the terminal portions of seedling corn roots favored the growth of excised corn root tips in an agar medium containing dextrose.

5. Extracts of germinated corn grains at higher concentrations injured excised corn root tips, producing hypertrophy of the cortex cells. At lower concentrations the extracts were beneficial.

6. Replacing the nutrient solution by fresh solution when the growth rate of excised corn root tips had decreased resulted in little or no benefit when a mineral nutrient solution containing dextrose was used, but caused renewed growth when the solution contained root extract, corn grain extract, or autolyzed yeast.

7. The significance of the experiments from the standpoint of a medium suitable for the continued growth of excised corn root tips is discussed.

Department of Botany
University of Missouri

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SECONDARY GROWTH BY MEANS OF A THICKENING RING IN CERTAIN MONOCOTYLEDONS

VERNON I. CHEADLE

(WITH PLATES II, III)

Introduction

It has long been known that in the arborescent and some herbaceous Liliflorae, as well as in a number of other groups of monocotyledons, there is an area of more or less radially placed secondary fibrovascular bundles imbedded in similarly oriented parenchyma, both arising from a cambial layer or zone. As a number of years have passed since any work of wide range has been done on these forms, it seems worth while to review, and in some degree to extend, the investigations which have been reported in the past.

The term "thickening ring" is used by SCOTT and BREBNER (**24**) as the English translation of the German "Verdickungsringe" and of the French "anneau de l'accroissement." There is considerable confusion in the literature concerning the actual connotations of these words; but there seems to be no doubt that the term as here used is at least a part of the full meaning of the word or words. A number of synonymous terms are used in this report, and are not to be confused with the "intrafascicular" cambial activity, which is more widely found in the monocotyledons.

In the course of the research, living plants of the following species were available from the Botanic Garden, Harvard University: Liliaceae—*Aloe saponaria* Haw., *Dasylirion quadrangulatum* S. Wats., *Sansevieria zeylanica* Willd., *Veratrum viride* Ait., and *Yucca glauca* ((Nutt.) in Fras. Cat.); Amaryllidaceae—*Agave americana* L. and *Furcraea pubescens* Tod. Preserved material of the stems of *Cordyline terminalis* Kunth and *Yucca aloifolia* L. was obtained from the Harvard collection of the Liliaceae. In addition to these forms, Professor I. W. BAILEY kindly permitted access to his prepared slides of various members of the Liliaceae: *Aloe arborescens* Mill., *Cordyline indivisa* Steud., *C. terminalis* Kunth, *Dasylirion serrati-*

folium Zucc., *Dracaena fragrans* Ker-Gawl., *D. goldieana* (Hort.), *D. hookeriana* C. Koch, *Nolina recurvata* Lem., *Yucca aloifolia* L., *Y. filamentosa* L., and *Xanthorrhoea arborea* R. Br. The names and authorities of all these plants were checked in the Index Kewensis (including the 1930 Supplement), except where they occurred in GRAY'S manual (**7**). The other plants mentioned were checked in the same publications.

Investigation

TECHNICAL PROCEDURE

Material from living plants was killed and fixed either in Carnoy's fluid or in formalin-acetic-alcohol, both prepared according to formulae given by CHAMBERLAIN (**5**). After proper treatment, following the general directions of CHAMBERLAIN, most of the organs were softened in a 50 per cent mixture of commercial hydrofluoric acid and 95 per cent alcohol. The material remained in this mixture for varying periods of time, depending upon its relative hardness. After subsequent washing in running water for 24 hours and then dehydrating, the various parts of the plants were imbedded in celloidin, following the procedure outlined by JEFFREY (**12**) and WETMORE (**26**), and finally stored in glycerin-alcohol.

Macerations of fresh or, when necessary, of killed and fixed material were made by the Cross and Bevan method (alternate treatment with hot sodium sulphite and chlorine water), or by hot saturated caustic potash followed by saturated chromic acid solution in water.

GENERAL DESCRIPTION OF SECONDARY BODY

The secondary body is composed of the tissues produced by a peripheral layer or layers of meristematic cells. That portion of the body of tissues on the inner side of the meristematic zone is characterized by vascular bundles imbedded in parenchyma, and that developed on the outer side (usually small in extent) of the dividing layer is characterized by parenchyma alone.

The general appearance of the secondary body has been described by a number of writers, who have discussed at length the elements of the xylem, and to some extent those of the phloem. Those treat-

ments which gave some insight into the general structure of the area under consideration usually dealt with single, or at most a few, species. Therefore the available material from eleven genera comprising eighteen species was studied in the attempt to discover what structures in the secondary body are common to all or a part of these species. Of these eighteen species, all but *Veratrum viride* have been at least mentioned in the literature.

Since this appears to be the first report of secondary growth in *V. viride*, the following is a brief description of the secondary body in this plant. The species in question is an herbaceous perennial with a short, thick, upright stem which gives rise to a large number of leaves whose sheaths form a false "stem." A cross section of the stem just below the point where the oldest functioning leaves are attached illustrates the structure of a normal short-stemmed monocotyledon. The large amphivasal bundles in the central area are imbedded in fundamental tissue, which is composed of cells with somewhat thickened walls, exhibiting many pits. The cortex contains the same sort of fundamental parenchyma. Separating these two areas, however, is a zone of radially oriented parenchyma in which are scattered small collateral bundles (fig. 5). This area, present only in the upper part of the stem, comprises a secondary body somewhat similar to that characterizing the better known species to be described later.

The secondary, collateral bundles consist of a few elements of xylem and phloem. Scalariform tracheids make up the xylem, and short sieve tubes and parenchymatous elements compose the phloem. It is unusual to find scalariform tracheids in secondary bundles; nevertheless the radial seriation of cells in this area indicates that true secondary activity is present in *V. viride*. Moreover, LINDINGER (15) cites not only his own observations, but also those of a number of others, in contending that comparable secondary thickening is present in many monocotyledons possessing a structural organization somewhat similar to *V. viride*. Thus, in the writer's observations, seriation in the stem of *Allium obliquum* L. represents secondary activity, although the peripheral bundles are imbedded in scarcely seriated "conjunctive tissue." (This term is used by EAMES and MACDANIELS (6) to describe the secondary parenchyma in

which the secondary bundles are imbedded.) The same situation, although less striking, is found in *Narcissus pseudo-narcissus* L., *Leucojum aestivum* L., *Hyacinthus orientalis* L., and many similar plants. In those species, however, the meristematic activity does not seem nearly so uniform as that found in *V. viride* and *Sansevieria zeylanica*. Only the latter two species are therefore included in the following discussion of the more typical secondary tissues. The presence of this rather anomalous type of secondary stem thickening in many of the bulbs, corms, or short aerial stems of the Liliaceae and the Amaryllidaceae may have some phylogenetic significance within these two families, but that subject will be considered at a future date.

It may be well to note here the means by which secondary bundles and the conjunctive tissue are distinguished from the primary body. RÖSELER (**22**) used the following diagnostic characters: (1) the paucity of the phloem elements in the secondary bundles, (2) the oval shape of the secondary bundles (fig. 1), (3) the more or less radial placement of these bundles, and (4) the radial orientation of the conjunctive tissue. In addition, I suggest that the short sieve tubes and lack of spiral or annular elements in these secondary bundles are valuable criteria. These characteristics seem to hold very well, except that the outer or peripheral bundles of the leaf traces often lack the annular and spiral elements also. The infrequent presence of two phloem groups in the secondary bundles (**13**) is not a good character, for these groups appear to arise from intimate anastomoses of these bundles. A combination of these characters, however, is usually sufficient to make identification of the secondary body reasonably easy.

The secondary area and its constituents were studied from a number of viewpoints. As will be indicated in a later section of this paper, the actual ontogeny of the secondary bundles and conjunctive tissue is fundamentally the same in the various plants examined. On the other hand, the structure of the mature secondary body may vary to such an extent that it provides a basis for identifying the species possessing such secondary tissues.

The placement of the inner group or ring of secondary bundles and the relative thickness of the walls in the surrounding conjunctive

tissue may vary considerably from that characteristic of the subsequently developed secondary body. Furthermore, this irregularity itself is of different magnitude in various species, since the inner area may be very compact or very loose in its construction. For example, in *Dracaena fragrans* it is very compact while in *Cordyline indivisa* the contrary is true, and in *C. terminalis* it may be either. About half the species examined have irregular areas (fig. 2) in the inner region of the secondary body and the other half have regular areas (fig. 3); that is, areas which do not vary from subsequently developed secondary tissues. Furthermore, two specimens of *Yucca aloifolia* exhibited two different types of inner ring formation, one being distinctly irregular and the other regular. Thus there is no characteristic of the first formed secondary tissue which is common to all plants possessing this development.

While there is some variation in the types of bundles present, either in the amphivasal or collateral forms, this seems to be a character which holds throughout the genera. For example, all the secondary bundles in the species of *Yucca* are characteristically collateral, and all those in *Dracaena* are amphivasal. The bundles are amphivasal in *Xanthorrhoea arborea*, but the phloem usually is nearer the outer (distal) portion of the bundles. The same is true of *Agave americana*, both in the rhizome and in the aerial stem; while in *Furcraea pubescens*, whose secondary bundles are small and contain few elements, there is a tendency toward variation from a typical amphivasal condition to one in which the tracheids do not quite surround the phloem. *Aloe arborescens*, contrary to PROLLIUS (**21**), has mostly amphivasal bundles.

There are only minor variations in the xylem parenchyma and in the conjunctive tissue in the species examined. All of the bundles contain at least a few xylem parenchyma cells. The walls of these cells are apparently always lignified; at least they stain with safranin. The position of the xylem parenchyma at the periphery of the bundles (also commonly found adjacent to the phloem) appears to conform more or less to generic lines. *Dracaena* possesses such an arrangement of the xylem parenchyma, as do most of the *Yucca* species, while the *Aloe* species do not. The apparent lack of conformity in *Dasylirion*, as well as in *Yucca*, may really be due to in-

ability to differentiate between the xylem parenchyma and the juxtaposed conjunctive tissue. In contrast to the cells of the latter, those of the former are usually slightly elongated and less broad in the radial and tangential directions. Also, where the walls in the conjunctive tissue are thickened, wall thickening is completed in the xylem parenchyma first. There appears to be considerable variation in the wall thickness of the secondary parenchyma area of the species investigated, but in general the thickness of the walls in this area is consistent throughout the genera. For example, as RÖSELER (**22**) commented, *Dracaena* has thick walled conjunctive tissue and *Yucca* has thin walled (except in certain concentric bands which are partly responsible for the formation of growth rings).

The xylem conducting elements are elongate, are often irregular in form, especially in the short stems, and frequently possess branched or forked ends. These conducting elements are surprisingly uniform throughout most of the species studied, with only *Aloe arborescens*, *Furcraea pubescens*, and *Dracaena fragrans* possessing a number of fiber-tracheids in addition to the normal tracheids. In the latter elements, the apertures of the bordered pit pairs are included, lenticular in outline and crossed, while in the former they are extended and more narrow. This is a convenient but arbitrary distinction. It agrees in general with the proposal concerning these elements made by BAILEY (**1**). Also in fiber-tracheids the borders are much reduced. Figure 9 shows the greatest thickness seen in the walls of fiber-tracheids. In all the elements considered, the pits are usually numerous and present on all walls. Apparently there is a correlation between the number of pits and their shapes. Thus the more numerous they are, the more oval are their apertures. Likewise, as is the common condition among tracheids, the thicker the walls, the narrower are the apertures. Among the well known monocotyledonous plants possessing secondary thickening, therefore, there is little variation in the types of conducting elements in the secondary xylem.

Exceptions exist, however, in *Veratrum viride* and *Sansevieria zeylanica*, for here scalariform tracheids occur in the secondary bundles. This situation may be true in all plants of similar habit which develop secondary tissues. At least in all those examined, similar tracheids were found.

Throughout the species examined, the phloem appears to be rather uniform in its construction and its types of elements; and although no detailed investigation of the phloem was made, most of this area undoubtedly consists of sieve tubes. They are short and have simple transverse sieve plates. Contrary to RÖSELER, the largest elements in the phloem appear to be the sieve tubes. Especially is this true in *D. fragrans*, where, in addition to terminal sieve plates, clusters of sieve fields were distinctly seen on the side walls. Thin walled parenchyma cells, identified by their nuclei, are present in all cases. The small size and position of some of these elements (in *D. fragrans*, for example) justify the conclusion that part of them are companion cells. Like the sieve tubes, neither of these elements elongates to any appreciable extent in the process of maturation. Although there are small variations in the number and size of the elements, there seems to be no obvious method of separating the genera on the basis of the phloem.

The parenchymatous tissue (secondary cortex) cut off to the outside varies in extent, there being but a small amount until the secondary meristem becomes a cambium of true initials, according to SCHOUTE (**23**). Extreme examples are *Furcraea pubescens* with a comparatively large amount of secondary cortex, and *Dasylirion serratifolium* with a meager development. The cells are usually rather oblong, with rounded ends in tangential view and rectangular in transverse and radial views. The only appreciable difference in this tissue throughout the species studied is in its extent, and that feature is not characteristic of genera.

Two conclusions may be drawn from the preceding description of the secondary body produced by a thickening ring in the monocotyledons: (1) While secondary areas have in common the presence of distinct vascular bundles separated by secondary parenchyma, variations occur in some features of the structure and arrangement of such tissues; (2) such variations may or may not be typical of a genus or even of a species.

GROWTH RINGS.—LINDINGER (**17**) discussed the cause of the concentric layering in the secondary tissue in monocotyledonous stems and reviewed the literature concerning this feature of the secondary body. He was able to distinguish three types of such growth layers, calling them annual rings (Jahresringe). CHAMBERLAIN (**4**), appar-

ently unaware of the work of LINDINGER or of HAUSMANN (8), pointed out growth rings in *Aloe ferox* and *A. pleuridens*. I have found evidence of similar layerings of structure in the secondary body of the stems in a number of plants. This layering in the secondary area is often rather obscure and an explanation of its formation is correspondingly difficult. The boundaries between zones are not distinct, since there are usually no obvious differences in cell sizes or types throughout any single growth ring. Furthermore, the zones may vary considerably in transverse view and may be very irregular in outline. These zones are called "growth rings" in preference to "annual rings," for it is not known whether they correspond to yearly increments of growth.

Dasylirion serratifolium, *Cordyline indivisa*, and *Yucca filamentosa* (given by LINDINGER as a type form) are examples of LINDINGER'S second type of ring formation, in which the differentiation of zones depends upon the relative number of bundles in alternating "dense" and "light" zones. In these species the rings are often composed of a single row of bundles. The growth rings in *Dracaena fragrans* are formed as a result of a similar differential frequency of bundles, but they are vague and difficult to identify because of the presence of consistently thick walled conjunctive tissue.

A compact group of bundles imbedded in thick walled conjunctive tissue alternating with more loosely arranged bundles surrounded by thin walled secondary parenchyma explains the concentric layering in *Yucca aloifolia*, *Cordyline terminalis*, and *Dracaena hookeriana*. The bundles are about the same size throughout. A structural pattern of this type is somewhat similar to that found by CHAMBERLAIN (4) in *Aloe ferox* and *A. pleuridens*, except that he described, as the diagnostic characteristics, bundles of a smaller size and parenchyma cells which had only "slightly thicker" walls than those of adjacent zones.

There seem to be, then, several types of such concentric layering in the secondary body. These types appear as a result of differences (1) in the size of bundles, (2) in the relative number of bundles per unit of area, (3) in the wall thickness of parenchyma cells, and (4) in the size and abundance of parenchyma cells. Any combination may occur. However, *Yucca aloifolia*, *Y. filamentosa*, *Cordyline in-*

divisa, and *C. terminalis* furnish evidence that any one plan, once established, appears to characterize a species but not a genus.

Origin and development of secondary body

The time at which secondary activity begins in the life history of any particular plant which eventually produces a secondary body has been discussed by Hausmann (8) and Wright (27). They agree that cambial activity begins in the hypocotyl and then "extends" up the stem. It arises similarly in *Dracaena* and also "extends," although more slowly, down into the roots.

The question of where the secondary meristem (cambium) originates in relation to the apex of the plant after it has passed the seedling stage is of greater interest in the present study. For many years (Millardet 20 to Carano 3) there has been a controversy over this question and the debated points have not yet been fully settled. Opinion has differed as to what constituted the cortex and the stele, and consequently the origin of the cambial zone was assigned by some workers to the pericycle and by others to the inner portion of the cortex.

Mangin (18) gave the name of cortex to all that tissue which arises from centripetal differentiation, with meristematic origin in the layer just beneath the epidermis, and that of central cylinder to the increment of tissue developed centrifugally. Baranetzky (2) found various types of development within the stem apex, and stated that the tissues of the cortex and the stele may be composed of cells of various origins. Carano (3), discussing the origin of the secondary activity, reported that there are no differences in the tissues of the stem which merit the name of cortex or stele until the secondary body is definitely present. Schoute (23), Hausmann (8), and Lindinger (15) produced evidence to show that secondary thickening is merely a result of the continuation of cell divisions in a restricted (peripheral) part of the primary body, that these cell divisions merely become periclinal in nature instead of haphazard, and that there is therefore no real distinction between the primary and the secondary meristematic activity. Scott and Brebner (24), on the other hand, were convinced that the secondary activity set in after the complete cessation of primary differentiation. They used

a vigorously growing stem for their investigations; but many other workers used comparable material in arriving at precisely opposite conclusions.

It is generally accepted that the pericycle is the outer layer of the stele, but if there is no clear differentiation of tissues in the stem tip, as brought out in the preceding discussion, then it seems impossible to locate exactly in which tissue the origin of the thickening ring takes place. Taking into consideration all the observations which have been made, including my own, the general conclusions may be drawn that a large part of the differentiation of primary tissues takes place before there are any secondary tissues laid down by the thickening ring, and that the region occupied either by the inner part of the cortex or by the pericycle gives rise to the cambial zone.

In regard to the type of cambium present, SCHOUTE (**23**) has shown it to be of an "Etagenmeristem" ("stage" or temporary meristem) form at first, which later is transformed into a true cambium, and he presented considerable detail to prove the validity of his assertions. It may be recalled that SCHOUTE's concept of an "Etagenmeristem" (a definition with which other invesitgators concur) is that certain cells become meristematic, divide several times and then lose this power, which is subsequently taken over by adjacent cells. MILLARDET (**20**) found in transverse view a cambial zone consisting of three to four rows of cells; HAUSMANN (**8**) and LINDINGER (**15**) agreed with SCHOUTE, and RÖSELER (**22**) claimed to have found that the cambial initials are strewn throughout the thickening ring. Neither the exact location nor a convincing description of the cambial initials could be found in the literature.

In attempting to describe the shapes of the cambial initials, therefore, one is confronted with the problem of locating them in the cambial zone. The slightly oblique tangential view of the thickening ring or cambial zone of *Cordyline terminalis* (fig. 8) shows that the cells differ from one another in shape. In *Dasylirion quadrangulatum*, however, a similar view (fig. 7) indicates that the cells resemble one another. According to SCHOUTE (**23**), the meristem becomes a layer of true cambial initials when cells are cut off to the outside; that is, when secondary cortical cells are produced. Consequently the view of *D. quadrangulatum*, at least, should be a picture of the true cam-

bial initial area, for there is a considerable amount of radially oriented secondary parenchyma outside the cambial zone. Hence in describing this area, one is sure to include the actual cambial initials. Reference to the figures shows that the cells in question in the tangential view may be fusiform, rectangular, truncate at one end and tapering at the other, or polygonal. Furthermore all such variations may occur in a relatively narrow region in a single plant (*C. terminalis*). This irregularity is reflected in the mature conjunctive tissue. When the cambial area is relatively homogeneous (*D. quadrangulatum*, *Agave americana*), however, all the cells appear to be more or less equilaterally polygonal. Longitudinal and cross sections of this region in *Aloe arborescens* (figs. 4, 9) indicate that the initials are rectangular in radial view and somewhat irregularly so in transverse. In *Agave americana*, *Yucca glauca*, *Aloe saponaria*, and *Furcraea pubescens* the cambial initials are also variable in form and intermediate in length between those of *D. quadrangulatum* and *C. terminalis*.

Judging from the species I have observed, therefore, there seems to be no justification for the general statement in EAMES and MACDANIELS (**6**, p. 248) in which the cambial cells are described as rectangular, or for that by STRASBURGER (**25**) picturing these cells as polygonal.

In general, the cell divisions in *C. terminalis* are in the longitudinal plane, although they may be also somewhat oblique. Those in *D. quadrangulatum* seem to occur in various directions. Nothing of final nature can be concluded concerning the divisions (and the cell contents) in these cells until actual living cambium is used in investigating this phase of the problem.

The history of the development of the desmogen strands[1] has been recorded by several workers. Reasonably complete observations by the writer for the most part confirm those made by SCOTT and BREBNER (**24**), HAUSMANN (**8**), CHAMBERLAIN (**4**), and others. A single daughter cell, resulting from subsequent divisions of cells originally cut off from the cambial initials, is the center of development of a

[1] SCOTT and BREBNER (**24**) used this term in preference to procambial strand. Since such an incipient bundle is produced by a secondary meristem, their term, originally used by RUSSOW as synonymous with procambial strand, is appropriate.

desmogen strand which eventually matures into a typical secondary bundle. This cell divides in an anticlinal direction to produce two or three rows; then these cells divide periclinally (fig. 12) and finally in haphazard fashion, although still in the longitudinal direction. Ultimately two or three daughter cells, one of which is usually a member of an adjacent radial row, normally enter into the formation of the bundle (**24**).

In most cases thickening of the walls of the tracheids on the inner (proximal) side of the bundle begins before all divisions are complete, but figure 9 (*Aloe arborescens*) indicates that this normal course of events is not always followed, for here the bundle is practically completed before any of the elements have even slightly thickened walls. Still more pronounced is the lag in thickening in *Yucca filamentosa*.

Many tiers of cells (as seen in longitudinal plane) aid in the production of a single bundle, for, as will be shown later, the conducting elements of the xylem elongate from fifteen to forty times their original length, while the xylem parenchyma and the phloem elements elongate little or not at all. RÖSELER (**22**) made counts of the mature tracheids in a great number of secondary bundles, and by making comparisons of their initial and mature lengths presented elaborate data to support his contention that only a single cell in any one tier eventually becomes a tracheid. SCOTT and BREBNER pointed out some fallacies in RÖSELER'S work, but admitted that not more than a "few" cells in any tier become tracheids. Similar data from my sections of *Dasylirion serratifolium* and *Agave americana* are comparable with those of SCOTT and BREBNER.

Even though there are but few (not over two or three) desmogen strand initials which become tracheids in any tier, the elements in the bundles at any one level mature in approximately the same order throughout the stem. Also the bundles in any species are generally of the same type, either collateral or amphivasal. The anatomical evidence indicates, therefore, not only that there appears to be a nicely timed mechanism which makes it possible for the bundles involved to differentiate from similar elements following approximately identical steps each time a new bundle is formed, but that whatever influence controls the differentiation of these bundles is active in a whole region of the stem rather than separately in each bundle.

As the cells in the desmogen strands are being produced through division, all of them become prismatic in form and sliding growth sets in. Longisections of the stem of *Aloe arborescens* (fig. 4), and especially of *Yucca aloifolia*, show that the intertwining of the tracheids makes it impossible to follow their development closely. To illustrate intermediate stages, therefore, and the final result of the elongation of tracheid initials by sliding growth, macerations were made of these bundles from several plants. Table 1 presents the maximum measurements of the mature tracheids.

TABLE 1

AMOUNT OF SLIDING GROWTH DURING MATURATION OF TRACHEIDS OF SECONDARY BUNDLES; MATURE LENGTHS ARE MAXIMUM MEASUREMENTS

PLANT	INITIAL LENGTH (MM.)	MATURE LENGTH (MM.)	RATIO OF MATURE TO INITIAL LENGTH
Agave americana	0.05	1.5	30
Aloe saponaria	0.075	1.8	26
Dasylirion serratifolium	0.05	2.0	40
Dracaena fragrans	0.05	2.0	40
Yucca glauca	0.075	2.5	37

There is some variation in the length of the mature tracheids, but little in the initials. Median measurements of twenty tracheids fall from 0.2 to 0.5 mm. below the maxima given in table 1, and measurements of minimum lengths indicate that fifteen is the smallest ratio. More measurements might well show greater variations.

The macerated material also revealed immature tracheids with thin walls, possessing single nuclei. These isolations, together with the results of counting the elements, give what RÖSELER and SCOTT and BREBNER considered as irrefutable proof that the tracheids arise from single cells and attain their maximum size by sliding growth. Considerable weight is added to that concept by the number of forked and branched tracheids which I isolated from the secondary bundles. Sliding growth also accounts for the connections of the secondary bundles to the old leaf traces, for elongating elements tend to follow along the latter if they happen to develop just below or above them.

PHYSIOLOGICAL CONTINUITY

MILLARDET, RÖSELER, SCOTT and BREBNER, and others have pointed out connections of the leaf traces to the secondary bundles. It is obvious that, if such bundles are to play an effective part in the transmission of water and nutrient salts from the roots to the leaves, and of elaborated foodstuffs from the leaves to the stems and roots, they must make close connections somewhere with one another and with the leaf traces. It should be kept in mind that physiological connection between the two types of bundles need not take place where the leaf traces quit the stele, for these traces curve inwardly before abruptly entering the cortical region. The union can be attained while the leaf traces are still near or at the periphery of the central cylinder. This point seems to be lost sight of, or perhaps merely taken for granted. Many have commented upon the course of the leaf traces throughout the arborescent stems of species similar to those considered here, and all report that the majority, if not all, of such plants have the same sort of fundamental plan just mentioned. At least the important traces of the leaves are thus arranged. Where the less important lateral traces "pass" into the leaf from the periphery of the stele, it is readily conceivable that there is a still greater possibility for connections to occur between secondary bundles and leaf traces. RÖSELER confirmed MILLARDET's report that there is no secondary activity while the leaf traces still function in their normal manner. On the other hand, SCOTT and BREBNER have shown that the outer, and therefore youngest, leaf traces are in fact completed by the secondary meristem; and according to CARANO, some of the "primary" bundles are actually secondary in their lower extremities.

My observations of the connections of the youngest common bundles to the secondary vascular tracts in *Agave americana*, *Aloe saponaria*, *Dasylirion quadrangulatum*, and *Yucca glauca* confirm the results of SCOTT and BREBNER, although the anatomical situation in these plants is not nearly so clear. Only *D. quadrangulatum* among these forms contained a large amount of secondary tissue.

The anastomosing of secondary bundles (fig. 11) has been noted by every investigator who has reported on this problem, and nothing more needs to be written concerning it except to repeat that the

greatest number of such anastomoses occur in the tangential direction. This would be expected, when the ontogeny of these bundles is recalled.

Moreover, there are undoubted physical connections between the old, non-functioning (from the standpoint of their original attachments) leaf traces and the secondary bundles. Good examples of this were found in all the plants examined, and figure 6 shows that this connection is indeed close and therefore may have some significance. The phloem and the xylem are in intimate contact. In the ultimate expression of this condition, these old leaf traces extend in a horizontal direction throughout most of the secondary body (figs. 1, 3). RÖSELER suggested that old leaf traces perhaps function to some extent as do vascular rays in the dicotyledons and the gymnosperms, for collenchymatous parenchyma (later becoming thick walled) is laid down around them as the cambial zone continues to increase the girth of the stem.

MANGIN (**18**), LINDINGER (**16**), and HAUSMANN (**8**) mentioned observations on the connections of the adventitious roots to the secondary body. In his report on the origin of adventitious roots and their influence on the anatomy of the stems in the monocotyledons, MANGIN concluded that the entire secondary area resulted indirectly from their production, but he was indefinite in regard to their actual origin. The writer has little to offer in the solution of that problem, for early stages in this development were not definitely identified. It seems safe, however, to infer from their positions (fig. 10) that they arise from the same cambial layer which produces the secondary tissues. They are often imbedded within that tissue, and in many sections of favorable material the spreading out of the bundles which connect with the vascular system of the adventitious root is evident. Perhaps this peculiar arrangement (réseau radicifère of MANGIN) makes it difficult to determine when a new root is in the process of formation. Such an arrangement has been seen just inside the cambial zone, and it is not beyond reason to interpret such a condition as the preliminary step in the formation of an adventitious root. The production of such roots has a considerable effect upon the appearance of the secondary body. When the roots are large, as in *D. quadrangulatum*, their influence on the secondary body is

correspondingly greater. The anastomosing among the "wing" bundles, as well as among these and the normally oriented secondary bundles, is obvious in longitudinal and in transverse sections of the stem.

From an examination of the anatomical picture afforded by these plants, therefore, it seems that there should be a continuous although at times devious pathway from the adventitious roots through the secondary bundles into the normally functioning leaf traces.

JACOB DE CORDEMOY (11) enumerated the functions of the secondary body. The bundles transport water, nutrient salts, and elaborated foodstuffs; the parenchyma serves as a storage mechanism, and both act as supporting tissues, the function of the latter varying with the number of bundles and the lignification of the elements. HOLM (9), confirming DE CORDEMOY'S work, found fatty oil in the rhizomes of *Cohnia flabelliformis*, sugar in *Yucca gloriosa*, and starch in several *Dioscorea* species, all in the secondary parenchyma. It seems safe, then, to infer that the secondary bundles carry elaborated foodstuffs.

To test roughly the physiological continuity within the plant from the standpoint of upward conduction, two whole plants each of *Agave americana*, *Aloe saponaria*, and *Yucca glauca* were carefully removed from their pots, and after being washed in water were placed in beakers of appropriate sizes containing a water solution of eosin. With the exception of two plants, *A. americana* and *Y. glauca*, which were left for forty-eight hours, they remained in this solution for twenty-four hours, with a fan gently playing over them to increase the rate of transpiration. Every two hours, hand sections were made of single leaves to note how far the eosin had traveled.

At the end of the test period, the eosin had reached the leaves in considerable quantity, so the plants were taken out of the solution and hand sections were made of the roots, rhizomes (*Yucca glauca*, *A. americana*), upright stems, and the leaves. As sections of all parts of other specimens of corresponding size had previously been made, it was easy to identify the extent of the secondary tissues. In the plants left in the eosin solution for forty-eight hours, all the secondary bundles and the peripheral group of primary bundles were stained. In the twenty-four hour plants, only the secondary bundles

in the rhizomes and in the aerial stems, together with the upper parts of the leaf traces, were stained. Macerations were then made of the stained portions of the stem, and the elements found checked in identity with the secondary elements which had previously been isolated from untreated plants.

The eosin solution therefore moved through the roots into the outer secondary bundles, then upward to where the secondary bundles unite with the leaf traces (at the beginning of the secondary activity), and thence into the leaf traces. The inward curvature of the latter to the central portion of the stele and their abrupt exodus to the cortex were well shown in this stained material.

These experiments are too few in number, perhaps, to form the basis for any generalization; but it appears that the shortest pathway to the leaves skirts the outer portion of the secondary area, and that consequently the remainder of the secondary bundles are brought into use only in "emergencies." Similar experiments are planned using plants possessing larger secondary bodies, in the hope of ascertaining whether the old leaf traces function as RÖSELER has suggested.

SECONDARY GROWTH IN ROOTS

Few critical observations concerning secondary activity in the roots of *Dracaena* were possible in the material available. Nevertheless a short discussion based for the most part on studies of *D. hookeriana* seems pertinent. The roots in this species have the usual generic type of primary structure, with a definite and conspicuous endodermis which stains deeply with safranin. The pericycle in the sections examined is thin walled and either one or two cells in thickness. The isolated strands of vessels and phloem in the pith are similar to those found in *D. fragrans* (fig. 14).

The actual onset of the secondary activity was not seen, but figure 13 indicates a rather early stage in the development. The endodermis is broken, but most of the cells are still in contact. One or two bundles have been produced, together with a small amount of conjunctive tissue. Figure 15 shows a later stage of this activity and there are now two rows of secondary bundles present. The endodermis is discontinuous and several of the cells probably have

been resorbed by the surrounding tissue (14). The radial placement of the bundles in later stages is similar to the condition found in the stem; hence the secondary growth in these roots appears to originate in the pericycle.

In *D. goldieana*, on the other hand, a situation was observed which parallels the descriptions of other species given by LINDINGER (14). The pericycle produced secondary tissues, and following early loss of its meristematic capability became, like most of its products, permanent thick walled tissue. Outside the bulged but unbroken endodermis, radial orientation of cells (indicating meristematic activity) signifies the appearance of a truly secondary cambial zone (14) similar to that in stems.

These observations tend to bear out CARANO'S opinion, later substantiated by MANN (19), that LINDINGER was too emphatic in his statement concerning the exclusively cortical origin of the secondary body in the roots of the species of *Dracaena*. It is impossible to interpret figures 13 and 15 in the light of LINDINGER'S concept. However, results based on a study of the situation in *D. hookeriana* do not necessarily weaken the generally accepted conclusion that, where there is a large secondary body present, most of the secondary tissue is produced by a generative zone originating in the cortex.

Summary

1. The structure of the secondary bundles, conjunctive tissue, and secondary cortex of certain monocotyledons is discussed, and the variations or similarities are enumerated. Genera usually may be characterized by the presence of either amphivasal or collateral bundles, and less markedly, by differences in structure of the conjunctive tissue. The elements in both the phloem and xylem and in the secondary cortex are strikingly uniform throughout most of the species examined. The first description of secondary growth in *Veratum viride* is presented.

2. Growth rings are found in a number of the genera, and depend upon differences in number and position of the secondary bundles, together with variation in wall thickness of the cells in the conjunctive tissue.

3. In the species examined there appears to be no sharp differenti-

ation into areas of distinctly primary or secondary meristematic activity. The cambial initials are of various shapes in tangential view. In the maturation of the secondary bundles, the conducting elements of the xylem attain their maximum length by sliding growth, the present observations confirming the results of certain earlier workers. All other elements of the bundles retain approximately the length of their initials.

4. Early secondary growth in the roots of *Dracaena hookeriana* is due to cell divisions in the pericycle.

5. Intimate structural connections between the adventitious roots and the secondary bundles, and between those bundles and the functioning leaf traces, aid in effecting continuity of the conductive tissues of the stem.

The writer expresses his sincere gratitude to Professor R. H. WOODWORTH of Bennington College and to Professor R. H. WETMORE for helpful suggestions and criticism throughout the course of this investigation.

BIOLOGICAL LABORATORIES
HARVARD UNIVERSITY
CAMBRIDGE, MASSACHUSETTS

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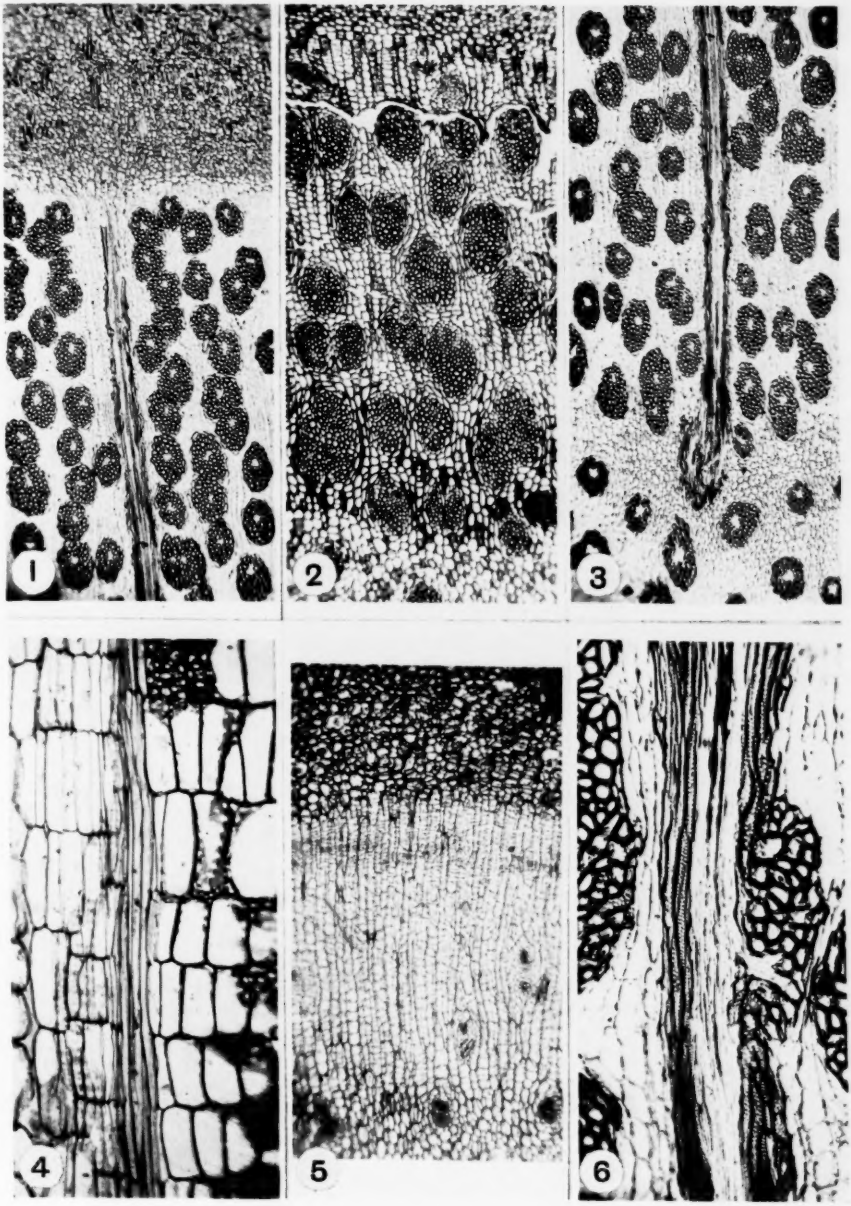
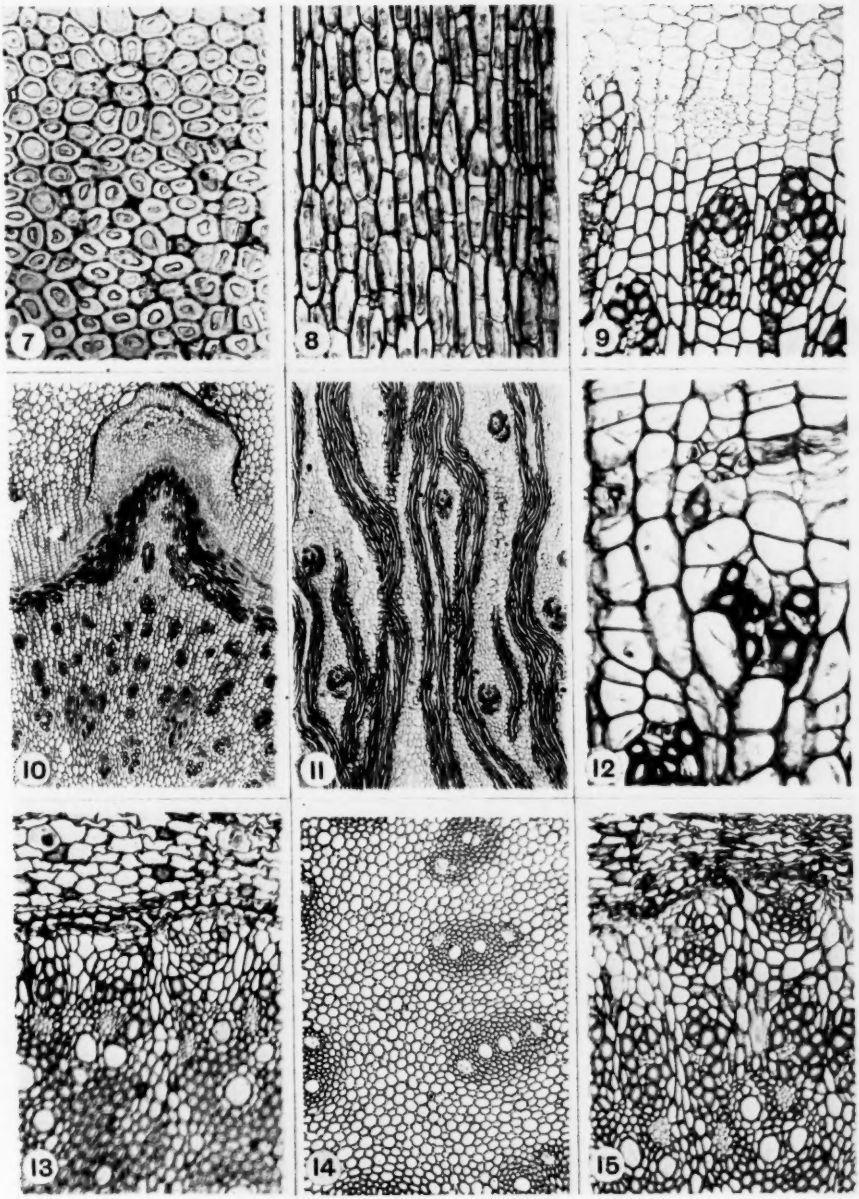


CHEADLE on SECONDARY GROWTH



CHEADLE on SECONDARY GROWTH

EXPLANATION OF PLATES II, III

PLATE II

FIG. 1.—*Dasylirion serratifolium* Zucc. Cross section of outer part of secondary body and inner part of cortex of stem. Note radial placement of secondary bundles. ×25.

FIG. 2.—*Yucca aloifolia* L. Cross section of secondary body of stem. Inner position of secondary body characterized by irregularly placed bundles and thick walled conjunctive tissue. ×30.

FIG. 3.—*Dasylirion serratifolium* Zucc. Cross section of inner part of secondary body and outer part of primary tissues in stele of stem. ×30.

FIG. 4.—*Aloe arborescens* Mill. Radial section of stem showing cambial region, immature desmogen strand, and heavily pitted conjunctive tissue. ×110.

FIG. 5.—*Veratrum viride* Ait. Cross section through secondary tissues of stem. Note paucity of secondary bundles. ×35.

FIG. 6.—*Dasylirion serratifolium* Zucc. Cross section of secondary body of stem illustrating connection of secondary bundle and leaf trace. Note intimate connection of phloem areas in center. ×110.

PLATE III

FIG. 7.—*Dasylirion quadrangulatum* Zucc. Slightly oblique tangential section of cambial region. ×90.

FIG. 8.—*Cordyline terminalis* Kunth. Section similar to preceding. Cambial initials included in vertical band of cells in center. ×90.

FIG. 9.—*Aloe arborescens* Mill. Cross section of stem showing cambial region, immature desmogen strand, and mature secondary tissues. ×85.

FIG. 10.—*Furcraea pubescens* Tod. Cross section of stem showing placement of adventitious root. Note secondary tissues of stem on inner side of attachment of root. ×28.

FIG. 11.—*Dasylirion serratifolium* Zucc. Tangential section of secondary body of stem illustrating anastomosing of secondary bundles. Leaf traces in cross sectional view. ×25.

FIG. 12.—*Furcraea pubescens* Tod. Cross section of stem showing immature desmogen strand. Fiber-tracheids in mature secondary bundle in lower part of figure. ×200.

FIG. 13.—*Dracaena hookeriana* C. Koch. Cross section of root showing secondary tissue under slightly bulged and broken endodermis. Small cells above vessels of primary body of stele indicate inner limits of secondary body. ×80.

FIG. 14.—*Dracaena fragrans* Ker-Gawl. Cross section of central portion of primary cylinder of root illustrating "pith bundles" of xylem and phloem surrounded by fibers. ×45.

FIG. 15.—Same as fig. 13. Later stage in production of secondary tissues with endodermis now discontinuous. ×85.

MORPHOLOGICAL AND CYTOLOGICAL STUDIES OF CALLA PALUSTRIS

MARGARET G. DUDLEY

(WITH PLATES IV, V, AND TEXT FIGURES)

Introduction

Although *Calla palustris* L., the wild calla lily or water arum, has received considerable attention from systematists, and has been of interest to cytologists, certain morphological and cytological features have not yet been accorded adequate treatment.

BUCHENAU (3) reported CASPARY as citing 272 cases of *Calla* as possessing two, three, or even four spathes on a single spadix, a fact noted also by HALSTED in 1874, and verified in connection with the preparation of this paper. KOSCHEWNIKOFF (12), writing on the development of the flowers of the Araceae, included a description of the inflorescence of *Calla palustris*. His statements have been verified, except those concerning a second type of placenta found in the lower flowers only.

ENGLER (5) monographed the Aroideae and, in a short paragraph on *Calla palustris* (6), stated that it is proterogynous, that the stamens dehisce sporadically after the stigmas have nearly all withered, and that the plant has therefore to rely principally on cross pollination (by means of insects). Later ENGLER and PRANTL (7) classified *C. palustris* as a close relative of *Lysichiton*, *Symplocarpus*, and *Orontium*. The validity of this arrangement will be discussed in a subsequent section. KRAUSE (13) repeated the substance of LIERAU'S (14) description of the root of *Calla*, amplifying it somewhat.

ARBER (1) traced (in the Araceae) the "course of differentiation from the family type to the specific type," and made several scattered references to *C. palustris*, with a number of figures illustrating the vascular bundles, the circles of adventitious roots, the ligular leaf sheath, and the spathe. METOLITZKY (16) described and figured the seed of *C. palustris*.

JÜSSEN (11) discussed the haploid generation of the Araceae, devoting considerable space to *C. palustris* and to *Symplocarpus*, but unfortunately omitted *Orontium* and *Lysichiton*, the other two genera of the Calloideae. The only point in which my observations do not agree with those of JÜSSEN is that the microspores show, in a number of cases, division of the generative nucleus while within the anther; they are therefore not binucleate as JÜSSEN has described them.

MARIE-VICTORIN (15) gave an account of *Calla palustris*, with a sketch of the flowering shoot, in which there are several errors: The filaments are much too slender, at least for the species as it occurs in Minnesota. The leaf venation is not correctly drawn, thus giving the impression that the "midrib" persists to the apex of the leaf. The inflorescence arises laterally, instead of being terminal, and the spathe has the appearance of encircling the spadix, which it does not do. The second leaf has no sheath, so that the peduncle apparently arises from the axil of the first leaf.

ERTL (8) discussed the development of leaf venation of the Araceae, including that of *Calla*, but the information contained in his publication has not been checked by the writer.

Material and methods

All the material was obtained in the state of Minnesota, from Itasca State Park, Cedar Bog in northern Anoka County, and from Grand Marais on Lake Superior, and consisted of spadices, rhizomes, and mature seeds. The spadices were fixed as soon as possible in either Navashin's or Carnoy's fluid, the latter solution being used for the stages which were young enough to admit of the possibility of finding reduction division in the pollen mother cells. The rhizomes, collected in late October, were covered with damp sphagnum and left in the open until thoroughly frozen (two–six weeks). They were then thawed gradually, planted in peat, and transferred to the greenhouse, where they began to grow almost immediately, blooming six weeks later, after producing three or four leaves. Some of the fruits, gathered in August, were placed in damp sphagnum, where the pericarps and mucilage disintegrated, setting free the seeds, which were then thoroughly dried before planting. Others were allowed to dry *in situ* and stored until needed.

All the material, after being dehydrated, was transferred to chloroform and imbedded in paraffin. The celloidin method was employed in the preparation of the rhizomes, because of their spongy nature. The seeds were soaked in weak hydrofluoric acid for three or four weeks, then imbedded according to the butyl alcohol method.

The sections of the root tips were cut 6–7 μ; those of the spadices 12 μ; those of the seed, seedling, and mature embryo 15 μ; and those of the rhizome 20–30 μ. Some of the rhizomes were stained with Delafield's haematoxylin, but safranin and fast green were used for the majority of them, and also for the seeds, seedlings, and growing points. Heidenhain's iron-alum haematoxylin was used for the embryos, crystal violet for the root tip sections, and iron-alum haematoxylin and crystal violet for the pollen mother cells.

Investigation

General description

Calla palustris is found in bogs and swamps of the northern hemisphere (**13**), occurring locally in moderate abundance in Newfoundland, eastern Canada, the Maritimes, Manitoba, and northern Saskatchewan, and sporadically in Alberta and British Columbia (only one record from British Columbia[1]). In the United States it is found as far west as Minnesota and as far south as Virginia. It is fairly abundant in central and northern Minnesota, except in the western portions, and blooms freely when not too shaded. The plant spreads over the mud by means of a branching sympodial rhizome, green in color, bearing circles of adventitious roots at the nodes. A bud is formed at each node, but very few of these develop into shoots. The leaves, which are confined to the current year's growth (the last few nodes), are, like the buds, alternate in arrangement. The inflorescence, as in other Aroids, is borne on the end of a terminal two-leaved shoot. A branch shoot arises in the axil of the penultimate leaf of this flowering shoot, and grows rapidly, producing a number of leaves (six–twelve normally) the first season. After remaining dormant during the winter, this rhizome bears two more leaves and an inflorescence, which terminates its growth. The plant

[1] Henry J. K., Flora of Southern British Columbia and Vancouver Island.

axis is then continued by another shoot, arising, like its predecessor, in the axil of the penultimate leaf of the flowering shoot (fig. 1). The rhizome, since it is usually submerged, is very spongy. Its large lacunae are filled with mucilage, and often contain raphide cells, which are much larger than those composing the lacunar walls. The cells containing the raphides are usually attached by one end to the small ones bordering the lacunae, and project therefrom into the mucilage-filled canals (fig. 8). The walls of the lacunae are one cell in thickness. In cross section they are dotted with scattered amphi-

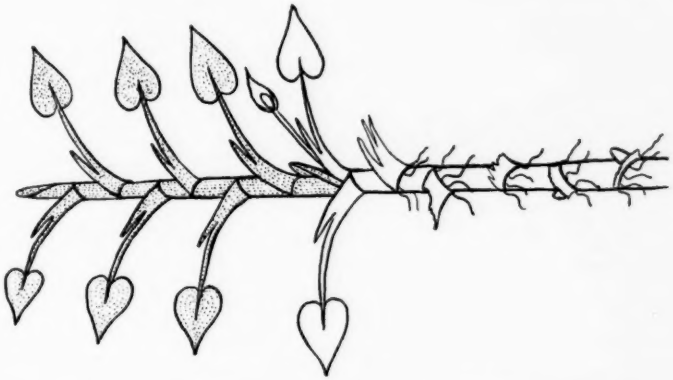


FIG. 1—Diagrammatic representation of sympodium of plant axis; continuation shoot stippled.

vasal vascular bundles, which frequently appear to be composed of two or three smaller bundles grouped together. The tracheae are few and very weakly lignified. Scattered laticiferous ducts, in connection with the vascular bundles, are evident in stained preparations (figs. 3, 8). Some of these vascular bundles form a ring at each node, outside which, in the young shoot, may be found a circle of adventitious root initials (fig. 9).

The adventitious roots are polyarch, with about seven small protoxylem vessels alternating with the sieve tubes near the periphery of the stele, and an approximately equal number of large metaxylem elements surrounding the small pith. The inner portion of the cortex is composed of radiating rows of regular, more or less

octagonal cells separated by large intercellular spaces, which are lacking between the smaller cells of the outer four or five layers.

The large, thin, cordate leaves have long spongy petioles concave on the adaxial surface. They have a divergence of one-half, and their vernation is convolute, successive leaves being rolled alternately right and left. The numerous well marked lateral veins diverge, one at a time, from the "midrib" and curve outward, parallel to one another, anastomosing just before reaching the margin of the leaf. In consequence the "midrib" gradually becomes narrower and finally disappears, its total length being about two-thirds that of the leaf blade. Each leaf possesses a ligular sheath (convolute in the bud), united with the petiole for approximately half its length and encircling the younger leaves (figs. 2, 4). The line of divergence from the rhizome being in the form of a spiral, with the ends overlapping, the nodes are in consequence not quite at right angles to the long axis of the rhizome, but are slightly slanting, the higher side of the node being alternately right and left (fig. 1).

The inflorescence appears in May (in Minnesota), when the plant has three or four leaves, two on the flowering shoot and one or two on the branch which is to continue the growth of the rhizome. As a rule there is not more than one inflorescence per year from each growing point, but there may be more than one spathe to a spadix (3).

Morphology and development of pollen grains

The anthers, which are very short in proportion to the filaments, are four-chambered and open extrorsely. The anther wall consists of three layers of cells: an epidermal, a palisade, and a tapetal layer (fig. 10), the first two of which are entirely normal in appearance and behavior.

The pollen mother cells undergo meiotic division while the archesporial cell is present in the ovule. They are large, easily stainable, and possess a conspicuous nucleolus. Previous to division the chromatin material of each nucleus becomes aggregated into a small, deeply staining sphere in close proximity to the nucleolus and very similar to it in appearance (11).

The meiotic divisions (figs. 11, 15) of the successive type take

place rapidly, and result in a considerable number of ellipsoidal pollen grains, so crowded as to be almost indistinguishable. These pollen grains are easily stainable throughout their development up to the point when they finally become separated from one another. They then apparently increase in size, without any appreciable increase of their plasma content, until the nucleus undergoes division. The pollen grain, while still uninucleate, possesses a large central vacuole, and its nucleus is often found near one end, imbedded in the peripheral layer of cytoplasm. Mitosis apparently takes place rapidly in this nucleus, since it was observed but rarely. The nuclei resulting therefrom, although at first identical, later become unequal in size, and are arranged in a line along the longitudinal axis of the pollen grain. Although JÜSSEN states that in *Calla*, division of the generative nucleus does not take place while the pollen grain is still in the anther, 2.5 per cent of the pollen examined by me was discovered to be trinucleate (fig. 16). As these pollen grains were not yet ready to be discharged, the percentage of trinucleate ones would doubtless be much higher at the time of dehiscence.

According to JÜSSEN, *Symplocarpus foetidus* has binucleate pollen, while that of *Zantedeschia aethiopica* and *Z. albomaculata* is trinucleate. In this respect, at least, no close affinity between *Calla* and *Symplocarpus* seems to be indicated.

The haploid chromosome number in *Calla*, as determined from the meiotic divisions (metaphase I, end view, and very late diakinesis) in the pollen mother cells is 18 (figs. 11, 15). The diploid number, as obtained from sections of the root tips, is 36 (fig. 13). Chromosome counts have not yet been recorded for any considerable number of the Araceae, but the basic number in the family appears to be 8 (9), *Spathiphyllum patinii* and *Acorus calamus* (unpublished data) being the only Aroids thus far discovered to have 9 as their haploid number. The data concerning the chromosome number in *Peltandra undulata* have apparently been misinterpreted. The haploid number is given by GAISER (9) as 22, on the authority of DUGGAR (4), but was apparently inferred from his writings to be the haploid number, since it is clearly shown by his illustrations to be the diploid number, if any. DUGGAR (4) states that there are about 22 chromosomes, referring to the first meiotic division, but neglects to state

whether the chromosomes occurred in *Symplocarpus* or *Peltandra*, or in both! The chromosome number for *Zantedeschia aethiopica*, as determined by OVERTON (18), is given as 16, while MICHELL (17) calculated it to be 12. OVERTON worked with greenhouse plants, however, and MICHELL with the native South African ones, which may account for the discrepancy between their results. It is obvious that much additional investigation is needed before any reliable conclusions can be drawn in regard to the basic chromosome number of the family, and as to what extent this criterion can be used as an indication of relationship and phylogeny.

In figure 16, illustrating the trinucleate pollen grain, may also be seen a smaller spherical body, which is one of the nuclei originally belonging to the tapetal layer already mentioned. The cells of this layer soon become dissociated, lose their walls, and form the periplasmodium typical of the Araceae (11), of which the nuclei only are still in evidence by the time the nucleus of the pollen grain is undergoing division. As may be seen from figure 16, the nuclei of the periplasmodium are slightly smaller than those of the pollen grains among which they are scattered, and contain a number of conspicuous chromatin granules, in addition to a nucleolus.

The mature pollen grains, in their natural condition, appear trapezoidal, with two lengthwise contraction grooves. When expanded by lactic acid and slightly stained with aceto-carmine, they are ellipsoidal and show no grooves. The exine is thick, subpunctate, and possesses three pores, one at the end of the grain slightly to one side and two near the other end, equidistant from its middle point and also from the first mentioned pore.

SEED AND EMBRYO

The seeds, of which two to fourteen are imbedded in the mucilage of each red berry-like fruit, develop from anatropous ovules[2] which bend outward from a raised circular placenta (fig. 7).

Double ovules sometimes occur, fused throughout their length (fig. 5), which develop into seeds containing normal embryos.

[2] The origin and development of the embryo and ovule have been omitted from this discussion, as a full description of the embryogeny of *Calla palustris* will be included in a later publication. Figures 14 and 19 illustrate typical stages in the development of the embryo.

The mature seed is brown, 3.7×1.6 mm. on the average, and pitted in regular horizontal rows. There are a number of dark spongy blotches at the chalazal end, possibly facilitating imbibition of water by the germinating seed (fig. 20). The pits are depressions in the epidermis, underlain by large subepidermal lacunae. The one-layered epidermis is composed of oblong palisade-like cells. The rest of the seed coat (developed from the outer integument) is formed

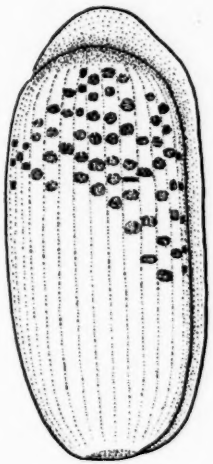


FIG. 20

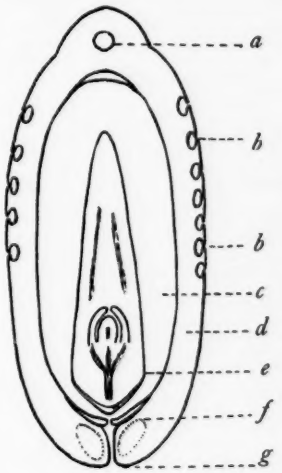


FIG. 21

FIGS. 20, 21.—Fig. 20, external view of seed showing raphe. Fig. 21, diagram of longitudinal section of seed showing embryo (*a*, vascular bundle; *b*, subepidermal lacunae; *c*, endosperm; *d*, seed coats; *e*, embryo; *f*, circum-micropylar canal; *g*, micropyle).

of about twenty rows of small isodiametric cells with intercellular spaces between them. The micropyle is practically closed, and a circular canal is plainly to be seen around it (fig. 21). The raphe possesses a large vascular bundle, and forms a ridge approximately 0.4 mm. wide, extending from the funicle to the chalaza, where it bends over and downward, expanding into a mass of tissue shaped like an inverted funnel. The abundant endosperm, which completely surrounds the embryo except at the micropylar end, is composed of starch-filled polygonal cells having no intercellular spaces. When

the hard testa is removed, the outer layer of the endosperm appears pale yellow and shiny, owing to the adherence of the crushed inner integument.

The mature embryo is green, lanceolate in shape (figs. 17, 18, 21), 2.5 mm. long and 0.6 mm. in its greatest width, central in position at the micropylar end of the seed, and often still attached to the suspensor (fig. 19).

The cotyledon, which forms the major part of the embryo, is thin at the base and sheathes the plumule. At this stage there are three longitudinal veins present which gradually converge, uniting a little below the solid apex of the cotyledon. The lateral veins sometimes unite before coalescing with the midrib, while in other cases they anastomose with the latter independently of one another.

The radicle is short and blunt, with a large central vascular strand, which divides near the apex of the hypocotyl (figs. 17, 21). Branches extend to the cotyledon, and to the leaves of the plumule, of which two are visible at this stage. The first leaf is inserted opposite the cotyledon, is curled inward at the apex over the second leaf, and usually possesses three longitudinal veins, similarly to the cotyledon, although cases were found in which there were four of these veins, two on one side of the midrib and one on the other. The second leaf is essentially similar to the first, and inserted not quite opposite it. The vascular supply to the first adventitious root leaves the main vascular strand immediately below the point where the latter divides into four branches, all of which arise at almost the same level. One of these branches supplies the midrib of the cotyledon, another all the veins of the first leaf, while the remaining two each supply one lateral vein of the cotyledon and part of the veins of the second leaf. Owing to the shortness, almost non-existence, of a hypocotylar region in the embryo, it is extremely difficult to distinguish with certainty the point of origin of the veins.

Germination of seed

The seeds were planted on damp sphagnum in petri dishes after having been soaked overnight. Those planted on May 5 germinated on May 11. Others planted January 17 (five months after ripening) germinated within four days. Seeds gathered in August germinated

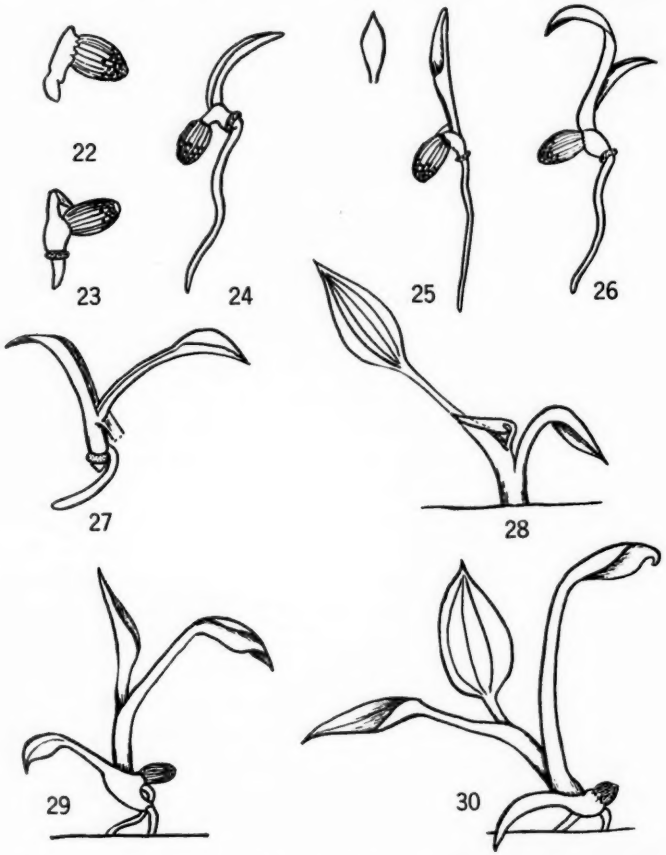
readily in October, after having been thoroughly dried, but would not germinate while still wet.

The radicle, which emerges through the annular canal, is green, massive, and blunt-pointed. It grows rapidly and turns downward, and the plumule is forced out of the seed by the extension of the base of the cotyledon (fig. 23). There is a spongy ringlike protuberance on the radicle, as shown in figures 23–27. The chief elongation of the embryo occurs above this structure, and is evidently due to growth in the basal part of the cotyledon, since in the several sections examined the upper portion still occupied its original position. Germination is evidently according to type B, as defined by BOYD (2), in which germination is hypogeal and there is a shallow collar edging the cotyledonary slit (figs. 24, 25). The three vascular strands of the cotyledon do not traverse this collar, but emerge through the "stalk" of the cotyledon close together while passing down the cotyledonary sheath, then converge to unite with the central vascular strand in the hypocotyl. The vascular supply to each petiole usually consists of three strands, which diverge, as do the cotyledonary ones, in cyclic order from the central mass of vascular tissue.

The leaves of the young seedling are similar in shape to those of the mature plant, but lack the ligular sheath. They have a divergence of one-half, their vernation is convolute, and each has a sheathing base which surrounds the younger leaves. The first leaf, which is usually small and not very well differentiated, emerges from the "collar" previously mentioned with its midrib on the side farther from the seed. Successive leaves are larger and more "*Calla*-like." Unfortunately none of the seedlings used survived beyond the four-leaved stage, so it is impossible to describe their subsequent growth (figs. 22–30).

In about half the cases studied the primary root did not develop at all, but the seedling derived its nourishment through an adventitious root, emerging from the central cylinder at a point immediately below the cotyledonary branches (fig. 12). In external view this root had its origin slightly above the spongy ring of tissue already mentioned, and marking the upper limit of the root sheath. In cases where the primary root grew and functioned, it broke through this sheath and lengthened rapidly. Unlike that of the seedling of

Zantedeschia aethiopica (3), the primary root of *Calla* bears no root hairs, but is itself short-lived, its place being taken by adventitious roots. This feature, according to BOYD, is an indication of "ad-



FIGS. 22–30.—Fig. 22, semi-diagrammatic drawing of seedling 2 days old; fig. 23, 4 days old; fig. 24, 7 days old; fig. 25, 8 days old; fig. 26, 11 days old; fig. 27, 13 days old (cotyledon broken off); fig. 28, 21 days old; fig. 29, 20 days old; fig. 30, 33 days old (same plant as no. 11).

vanced morphology" of the seedling; on the other hand, a simple tubular cotyledon, such as that possessed by *Calla*, is to be regarded

as a "primitive characteristic." *Zantedeschia* (*Richardia*) *elliottiana* is the only rhizotomous Aroid discussed by BOYD, and as that is done in a rather sketchy manner, one is not able to make comparisons. The germination of *Zantedeschia* (*Richardia*) *aethiopica*, as described by BUCHENAU, and also as attempted by the writer, seems to be very similar to that of *Calla palustris*, and if carried through might prove to be another link in the chain of evidence binding *Calla* and *Zantedeschia* together.

Systematic position of Calla

One of the more revolutionary changes in the classification of flowering plants, as set forth by HUTCHINSON (10), concerns the Araceae, and therefore a short résumé of the history of the classification of the Araceae is here given.

BENTHAM and HOOKER (1862–1883) place the Araceae together with the Pandanaceae, Cyclanthaceae, Typhaceae, and Lemnaceae, in series 5 (Nudiflorae), while the Liliaceae are in series 3 (Coronarieae). In ENGLER and PRANTL (1924 edition) the Araceae and Lemnaceae form the 7th "Reihe" (Spathiflorae), while the Liliaceae are placed in the 9th. LOTSY (1911) derives the Araceae, together with the "Spadiciflorae" (Lemnaceae, Cyclanthaceae, Palmae, Pandanaceae, Sparganiaceae, Typhaceae), from the Piperales, and the Liliaceae from the hypothetical "*Pro-ranales*." BESSEY (1915) places the Araceae in the Strobiloideae (ovary superior) and apparently considers them to be derived after the Liliaceae, although this point is not clear. WETTSTEIN (1924) unites the Araceae with the Palmae and the Cyclanthaceae in order 8 (Spadiciflorae), while the Liliaceae are in order 2 (Liliflorae). MEZ (1926) in his sero-diagnostic chart places the allied Araceae and Lemnaceae on the same branch as the Palmae and Cyclanthaceae, but the Liliaceae alone on another branch of the monocotyledons, and further from the main stem. JÜSSEN (1928), after reviewing several systems of classification, agrees with ENGLER in considering the Araceae and Lemnaceae as forming an independent order, and also cites several points of similarity between the haploid generations of the Araceae and the Helobiae (trinucleate pollen grains, periplasmodium, endosperm formation), evidently considering the Liliaceae to be unrelated to either.

HUTCHINSON (1934) places the order Arales (Araceae and Lemnaceae) in the subphylum Corolliferae (with a corolla-like perianth), and derives them directly from the Liliaceae through the tribe Aspidistreae. The Helobiae are all included in the subphylum Calyciferae (perianth biseriate). If this arrangement be correct, the cytological similarities observed by JÜSSEN must be due to parallelism.

HUTCHINSON places *Calla palustris* in the tribe Calleae (number 8 of the series of 17 tribes constituting the family), and it is therefore considered to be more highly evolved than *Acorus*, *Lysichiton*, *Orontium*, and *Symplocarpus* but less so than *Zantedeschia*, *Aglaonema*, *Arum*, and *Arisaema*. According to HUTCHINSON, the more primitive Araceae are those with a poorly developed or leaflike spathe, and hermaphrodite flowers possessing a perianth. He states (10, p. 119), "The more highly evolved Araceae would have unisexual flowers, an increasingly protective spathe, and owing to reduction, some part of the spadix would become barren." An examination of *Calla* readily shows that it is intermediate between these two extremes, and therefore correctly placed with regard to the principles just enunciated.

ENGLER (**6**, p. 122) likewise places *Calla palustris* in the tribe Calleae, but unites it with the tribe Symplocarpeae (*Lysichiton*, *Symplocarpus*, and *Orontium*) into the subfamily Calloideae, one of whose characteristics is said to be the possession of a creeping underground rhizome [elaborated upon by KRAUSE (13) and which unfortunately, as shown by ROSENDAHL, is not present in either *Symplocarpus* or *Lysichiton* (**19**)]. Another characteristic stressed by ENGLER is the presence of "simple or unbranched latex ducts in connection with the phloem of the vascular bundles" (**19**, p. 138). This characteristic, however, is not common to the four genera forming the Calloideae, but occurs in *Calla* and *Orontium* only, thus casting further doubt upon the validity of ENGLER'S disposition of these genera.

HUTCHINSON distributed the Calloideae of ENGLER among the three tribes Orontieae (*Lysichiton* and *Orontium*), Dracontieae (*Symplocarpus*, *Dracontium*, *Echidnium*, and *Dracontioides*), and Calleae (*Calla*), the last being the most advanced and distinguished

from the other two by the absence of a perianth (a characteristic shared by the Monstereae). The Dracontieae are separated from the Orontieae by reason of the possession of a well differentiated spathe (10).

KRAUSE also distinguishes *Calla* from the other three genera (Symplocarpeae) through the absence of a perianth, coupling with this characteristic the presence of endosperm and parallel lateral veins.

A comparison of the four genera seems to show more differences than similarities, and would therefore lead to the conclusion that they cannot be combined into one subfamily, and that HUTCHINSON'S arrangement (in the order of evolutionary progress) is the most satisfactory one yet proposed.

Summary

1. *Calla palustris* spreads over the ground by means of an alternately branching sympodial rhizome, which bears numerous adventitious roots at the nodes.

2. Each growing point is active for two seasons, producing several leaves the first year and two more the next spring, followed by an inflorescence, which terminates the growth of that branch of the rhizome.

3. Each principal plant axis is continued by the production of a branch in the axis of the penultimate leaf of the flowering rhizome.

4. The leaves are thin, bright green, and cordate, with ligulate sheathes. Their vernation is convolute, and they are rolled to right and left alternately.

5. The ovary is unilocular, and contains from two to fourteen anatropous ovules. Double ovules, fused longitudinally, sometimes occur.

6. A periplasmodium is formed in the anther. The pollen grains are apparently trinucleate before dehiscence occurs.

7. The seed is albuminous, and possesses a hard seed coat. The embryo is central, and germinates without a period of rest if the seed has been thoroughly dried.

8. Germination is hypogeal. The base of the cotyledon elongates, forcing the radicle and plumule down into the substratum. The

cotyledonary slit is edged by a collar, through which the plumule emerges. The vascular strands of the cotyledon are three in number, and do not traverse the collar.

9. The primary root sometimes develops, but more often does not, when its place is taken by an adventitious one. This adventitious root is very noticeable, even before germination.

10. The leaves of the young seedling have no ligular sheath, but resemble adult leaves in all other particulars.

11. The haploid chromosome number, as determined from pollen mother cells at metaphase I, is 18. Metaphase plates of root tips show 36 chromosomes.

The writer wishes to thank Dr. C. O. ROSENDAHL and Dr. F. K. BUTTERS, of the University of Minnesota, for their continued interest and stimulating advice, and Mr. A. O. DAHL for his technical assistance.

DEPARTMENT OF BOTANY
UNIVERSITY OF MANITOBA
WINNIPEG, CANADA

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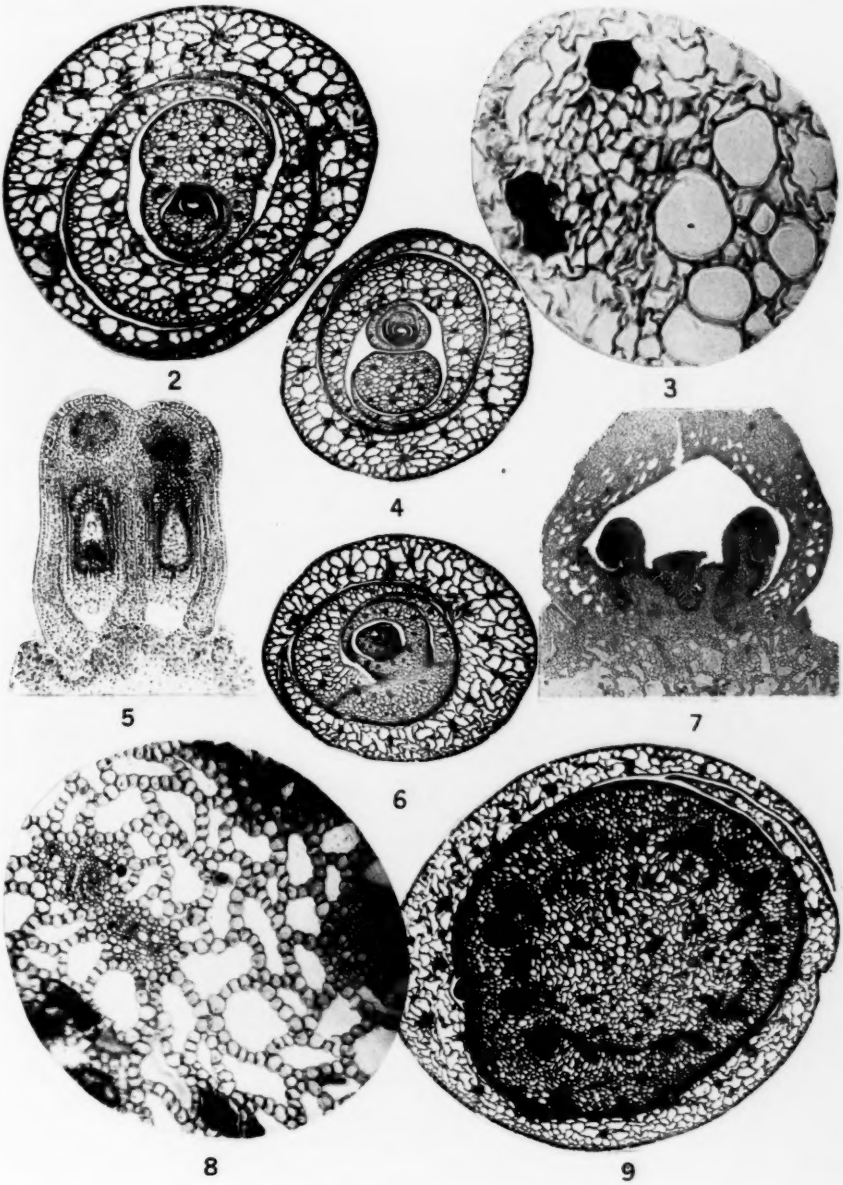
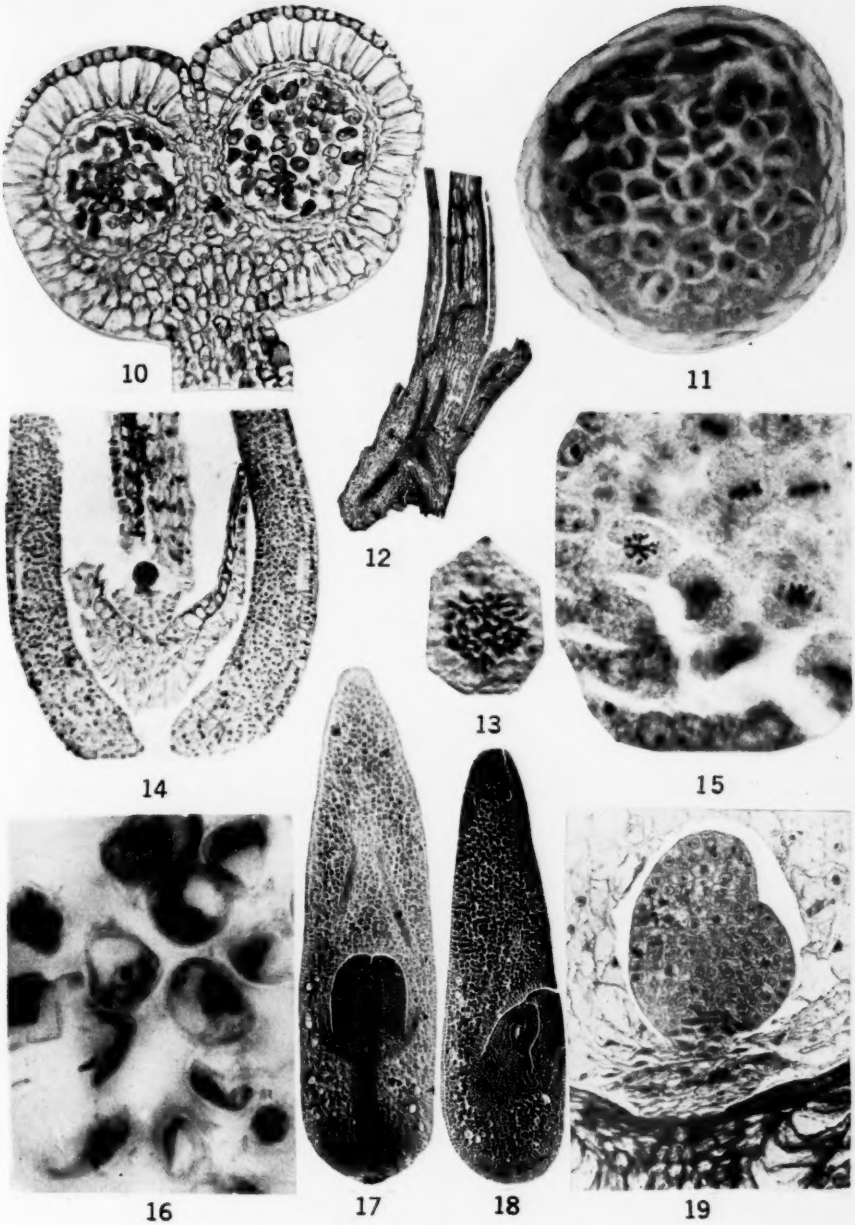


DUDLEY on CALLA PALUSTRIS



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EXPLANATION OF PLATES IV, V

PLATE IV

FIG. 2.—Cross section of growing point showing four leaves.

FIG. 3.—Detail of vascular bundle in rhizome showing laticiferous ducts.

FIG. 4.—Cross section of growing point, slightly below level at which fig. 13 was taken.

FIG. 5.—Double ovule, fused longitudinally.

FIG. 6.—Cross section of growing point showing node.

FIG. 7.—Longitudinal section of young ovary showing two ovules.

FIG. 8.—Cross section of rhizome showing vascular bundles, raphides, and mucilage.

FIG. 9.—Cross section of node showing bud in axil of sheathing leaf.

PLATE V

FIG. 10.—Longitudinal section of stamen.

FIG. 11.—Cross section of anther sac showing meiotic division in pollen mother cells.

FIG. 12.—Longitudinal section of 21-day old seedling showing first adventitious root, junction of cotyledon, and vascular supply to cotyledon.

FIG. 13.—Cell of root tip showing metaphase stage of mitotic division.

FIG. 14.—Multicellular proembryo showing nucellar cap.

FIG. 15.—Detail of meiotic division in anther sac.

FIG. 16.—Mature pollen grains showing one trinucleate grain and one of the nuclei of periplasmodium.

FIGS. 17, 18.—Longitudinal sections of mature embryos from ripe seeds.

FIG. 19.—Multicellular proembryo, with suspensor.

STRUCTURE OF PALEOZOIC SEEDS OF THE TRIGONOCARPALES

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 476

CHARLES L. DEEVERS

(WITH THIRTY-SIX FIGURES)

Introduction

Much interest has been shown in the seeds of the Paleozoic era since the discovery that many of the fern-like leaves of that period belong to the gymnospermous Cycadofilicales as well as to the true ferns. BRONGNIART grouped these seeds into two classes, the Platyspermae and the Radiospermae. SEWARD classified the 29 genera of these seeds into three orders, the Lagenostomales, the Trigonocarpales, and the Cardiocarpales. The Lagenostomales and Trigonocarpales include BRONGNIART'S Radiospermae, and the Cardiocarpales his Platyspermae. These Paleozoic seeds show affinities with the seeds of the cycads.

More than 65 species of *Trigonocarpus* have been described during the past hundred years, mainly as seed casts. The structural details of scarcely more than half a dozen have been given. FIEDLER (3), KRICK (4), and SEWARD (8) have summarized the literature of this group.

Six species of the Trigonocarpales are described in this paper, five of them belonging to the genus *Trigonocarpus*. Following the suggestion of SCOTT and MASLEN (10), the term *Trigonocarpus* has been used rather than *Trigonocarpon*, which was used by WILLIAMSON and NEWBERRY.

The specimens of *Trigonocarpus* studied were collected at Petit Jean Mountain, 12 miles southwest of Morrilton, Arkansas, in Cedar Creek Canyon, about three-fourths of a mile below the waterfall of the same name. They were found literally by the bushel in huge boulders which had fallen down from the eastern side of the cliff. The seeds occur in a hard, gray sandstone in a layer about 3 feet thick which overlies a thick bluish shale. This sandstone layer is 100

feet or so above the bottom of the canyon, and approximately 200 feet below the edge of the cliff. The same stratum appears again on the opposite side of the mountain, where these specimens are numerous. The geologic horizon is upper Atoka, about 150 feet below the Hartshorn sandstone which caps Petit Jean Mountain. The Atoka and the Hartshorn correspond to the Pottsville in age, and therefore represent the Lower Pennsylvanian strata (2). One specimen of the Trigonocarpales was collected by Mr. N. J. Gillett in the Strip Mine of the Northern Illinois Coal Company at Wilmington, Illinois. It was found above coal no. 2, which forms the basis of the Allegheny group of the Pennsylvanian system in Illinois.

For detailed studies of internal structure and other anatomical features of these casts, sections of the material were made by means of the diamond saw and carborundum powder. After carefully polishing the faces of the sections, a thin film of canada balsam was spread over them, and they were examined by reflected light. Thin sections of *Trigonocarpus* failed to show any cellular detail, so descriptions are based mainly on surface views. A total of 150 specimens was available, and of these approximately 100 were sectioned. Thin sections and peels were made of the Illinois specimen by the use of the celloidin film method. Histological details were found in these.

Among the material available there are specimens representing at least two previously described species. These are *Trigonocarpus dawesi* Lindley and Hutton and *T. noeggerathi* Brongniart. The same species have been described by several investigators from material obtained both in Europe and America. The remaining species are considered to represent three new species and one new genus and species. None of the specimens of *Trigonocarpus* shows any cellular details, but certain structural features are evident from the regular occurrences of carbonized remains of membranes.

None of the seeds upon which this investigation is based was found directly attached to other plant organs, but several fragments of stems and pith casts of Cordaites were found associated with them. Near the locality where the seeds were found, many casts of *Stigmaria ficoides*, some species of *Calamites*, and a few stems of *Lepidodendron* were present. The seeds show no evidence that they were transported or stream worn; they appear to have been fossilized where they fell.

Description of species

Trigonocarpus dawesi Lindley and Hutton (figs. 1–8, 12)

Fifty seeds of this species were investigated, and 35 of them were sectioned. They are of large size, varying in length from 3.5 to 5.5 cm., oval in shape, nearly circular in cross section, and from 1.7 to 2.7 cm. thick. They bear on the surface three nearly equidistant salient ridges or wings. About half of the specimens are winged

FIGS. 1–8.—Fig. 1, *Trigonocarpus dawesi* Brgt., nearly median longitudinal section: *m*, micropylar end of cast; *p*, pollen chamber with top broken and resting on megaspore membrane; *a*, darker layer, the sclerotesta; *b*, carbonized remains of inner flesh; *c*, megaspore membrane-nucellus remains. Figs. 2–4, serial sections from median to outside of same cast. Fig. 5, median longitudinal section of another cast of same species; megaspore membrane-nucellus remains crushed and invaginated. Figs. 6–8, *T. dawesi* Lindley and Hutton: 6, view of basal end showing foramen partly filled with carbonized remains of vascular tissue; 7, side view showing one of three ridges; 8, micropylar end with part of wing still intact at upper right. Note tendency that many specimens have of splitting into three valves.

(fig. 8) and half bear ridges only (figs. 6, 12). These wings and ridges extend the entire length of the seed, from about 5 mm. above the foramen to the micropyle. The wings are approximately 1 mm. thick, and increase in width from near the foramen to a point about 1 cm. from the micropyle, where they are 4 to 5 mm. wide. The ridges are uniform in size.

The sclerotesta is evident in many of the sections as a darker layer, the outermost layer of the cast, and is about 1 mm. in thickness (fig. 1 *a*). It is also evident in the winged specimens (fig. 8) and probably in those having ridges (fig. 12). In many of the specimens the sclerotesta is sharply pointed in the micropylar region; in others it is less pointed or broken. The basal end is rounded, and near its center is a conspicuous foramen (fig. 6).

The inner flesh is represented by a thin carbonized membrane just inside the sclerotesta (fig. 1 *b*). This membrane extends over the entire inner part of the seed from the basal disk to the micropyle, although it is often broken and crushed (fig. 5). In figures 1–4 it is shown in about its original position.

There is no indication of a nucellus separate from the megaspore membrane and the pollen chamber. The latter is evident in many of the specimens as a truncated cavity about 3 mm. high, and in the best specimen is 1 cm. broad at its base (fig. 1 *p*). The membrane inclosing this structure is broken and shrunken, as are the other membranes. The vascular organization is shown poorly, except as a carbonized mass in the foramen at the base of the seed (fig. 6). The nucellus-megaspore membrane is much more prominent than the other membranes; apparently the nucellus had a definite vascular system which is carbonized and included in this complex.

The megaspore membrane appears relatively thick, probably because the nucellar remains are also in contact with it. It is in about its original position in figures 1–4, where it surrounds the cavity previously occupied by the megagametophyte. The megaspore membrane usually is invaginated as seen in figure 5.

The winged specimens represent casts of forma beta as designated by ARBER (1); that is, those which have the sclerotesta as the outermost part. It is possible that the ridged specimens represent forma gamma casts (casts of the inner portion of the sclerotesta only). In the Arkansas material both the ridged and the winged specimens show a foramen at the base, and a darker layer about 1 mm. thick as the outermost layer of the cast. This darker layer is the sclerotesta. It is not known whether the sclerotesta represents the former thickness of this layer, but the sclerotesta of the known Trigonocarpales is somewhat less than 1–4 mm. in thickness. Usually the

wings are broken off in removing the seeds from the inclosing matrix, and the seeds have a tendency to split into three nearly equal valves (fig. 8). The question arises whether the specimens possessing wings are to be considered as belonging to the same species as those having ridges, or distinct from them. Those specimens which bear wings along the entire length of the seed closely resemble *Trigonocarpus bertholletiformis* (*T. bertholletiforme* Foster; **5**, pl. 42, figs. 12, 12 *a*); but after allowing for a degree of variation among the specimens available, it seems more probable that they are variants of a single species.

Trigonocarpus noeggerathi Brongniart (figs. 9–11, 26)

Fifty seeds of this species were studied, and about 35 were sectioned. They were not so large as those of *Trigonocarpus dawesi*. They vary in length from 2 to 3.4 cm., are ovate, and are nearly circular in cross section. In breadth they have a maximum diameter of 2 to 2.4 cm., and bear on the surface three nearly equidistant wings which extend from near the hilum to the micropyle. These wings are not so prominent as those of *T. dawesi*, and a large number of the seeds tend to break into three equal valves along the line of the wings, in the same manner as the former species.

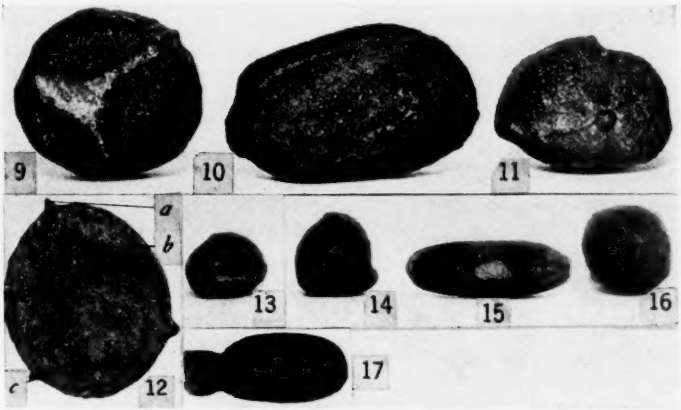
The sarcotesta is represented by a thin carbonized film uniformly thick over the entire seed; and as in the former species there is no indication of the thickness which this part of the integument may have had in the living state.

The sclerotesta is evident, as in *T. dawesi*, as a darker layer about 1 mm. in thickness. It is less pointed in the micropylar region than it is in the former species, and has a blunt appearance (figs. 9, 10). The rounded base has the foramen as has *T. dawesi* (fig. 11).

The inner flesh is represented by a thin carbonized membranous structure immediately inside the sclerotesta (fig. 26 *b*). This membrane extends over the entire inner part of the seed from the basal disk to the micropyle. Often it is broken and displaced.

There is no indication of the nucellus other than the megaspore membrane and the pollen chamber. Since the innermost membrane of these casts appears much more conspicuous than the membrane representing the inner flesh, it is probable that the nucellus, with its vascular tissue (**9**), is not separate from the megaspore membrane.

The pollen chamber is evident in many of the specimens as a truncated structure just above the megaspore membrane. The membrane about the pollen chamber is shrunken and collapsed in figure 26, and is not so large, proportionately, as the same structure in *T. dawesi*. The vascular organization is poorly shown, except as a carbonized mass in the foramen. What may have been the extent of the vascular tissue originally present is not indicated, unless the



FIGS. 9–17.—Fig. 9, *Trigonocarpus noeggerathi* Brgt., micropylar end showing three wings, or salient ridges. Fig. 10, side view, micropylar end to left, showing part of two wings. Fig. 11, basal view showing foramen partly filled with carbonized remains of vascular elements. Fig. 12, *T. dawesi* Brgt., cross section at middle of cast: *a*, one of three ridges; *b*, folded and shrunken remains of inner flesh; *c*, megaspore membrane-nucellus complex. Fig. 13, *T.* **costatus** sp. nov., micropylar view showing three primary ridges with about 20 smaller ones between them. Fig. 14, basal view showing characteristic splitting. Fig. 15, side view, basal end to left. Fig. 16, micropylar view showing slightly pointed apex. Fig. 17, *T.* **serratus** sp. nov., side view, basal end to right. Three of the six divisions of cuplike structure of summit seen at micropylar end to left.

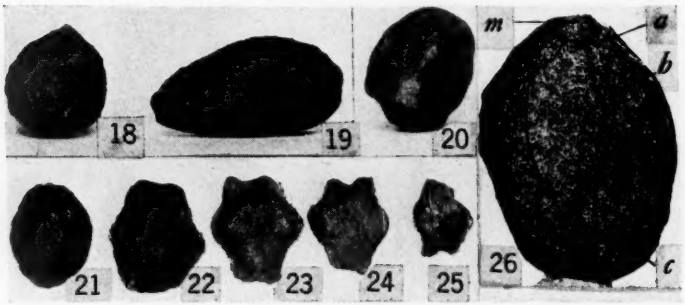
thickness of the layer representing the megaspore membrane-nucellus includes the vascular tissue of the nucellus (10).

These casts vary considerably in size and shape. Again it is possible that more than one species may be represented in this collection, but until better preserved specimens are described it seems best to consider them as belonging to one species.

Trigonocarpus **ouachitensis** sp. nov. (figs. 18–25)

DIAGNOSIS.—A radially symmetrical obovoid seed, flattened at base and circular to hexagonal in cross section. Sclerotesta (length 2 cm., width 1 cm.) produced into a sharp apex, and bearing six ribs (three principal sharp angled and three less prominent). Ridges flatten out equally near the middle of the seed to form an irregular to regular hexagon at the base. Sarcotesta present only in the form of a thin carbonized membrane.

This species is represented by 33 specimens, 15 of which were sectioned. It is much smaller than *Trigonocarpus noeggerathi*, and is



FIGS. 18–26.—Fig. 18, *Trigonocarpus* **ouachitensis** sp. nov., basal view showing hexagonal form of sclerotesta about base. Fig. 19, side view, micropylar end to left; three of the six ridges apparent. Fig. 20, micropylar end with three major and three minor ribs; apex broken off. Figs. 21–25, serial sections from base to near the micropyle. Fig. 26, *T. noeggerathi* Brgt., near median longitudinal section: *m*, micropylar end; *a*, sclerotesta; *b*, carbonized remains of inner flesh; *c*, megaspore membrane-nucellus, much shrunken.

characterized by the seeds being of comparatively uniform size. While there is little evidence that the three principal ridges were prolonged into a micropylar tube, it is possible that this was the case. The three less prominent ribs end at the micropyle, and in many of the specimens the micropylar end is broken off (fig. 20). There is no evidence in the matrix which surrounded the casts of the presence of a micropylar tube, but it is only after well preserved structural specimens of *T. parkinsoni* were studied that this structure was found in that species (10). The base of the seed is somewhat dome-shaped, resembling in this respect the base of *T. oliveri*

Scott and Maslen (10). It differs from *T. oliveri* in that the base is not so broad, proportionately, and in tapering more sharply toward the micropyle. The base is distinctly hexagonal in most of the specimens. SALISBURY (7) thinks that *T. oliveri* has eight ridges, and that it is not a true *Trigonocarpus*.

There is little structure preserved in these specimens. The carbonized remains on the outside of the casts are apparently much thicker than in the former two species, indicating that the sarcotesta was of much greater thickness in this seed. Figures 21 to 25 show a broken, carbonized, membranous structure, which probably represents the megaspore membrane-nucellus remains. The nucellus was free to the base, and has vascular tissue present.

Trigonocarpus **costatus** sp. nov. (figs. 13–16)

DIAGNOSIS.—A radially symmetrical seed cast, elliptical in longitudinal section and circular in cross section. Sclerotesta (length 10 to 20 mm., width 7 to 10 mm.) slightly pointed at apex, rounded at base, and bearing on the surface three faint, equidistant ridges which extend from the base to the micropyle. Between the ridges occur about 20 faint striae, equally spaced, and running the entire length of the seed (figs. 13, 16).

This specimen resembles *Trigonocarpus multicarinatus* Newberry (5) in that it possesses a number of striae. These ridges are not so prominent as those of the latter species, and the larger ridges are not prolonged into the raised center of a cupped summit as in *T. multicarinatus*. *T. costatus* is slightly pointed at the micropylar end and not truncate as is *T. multicarinatus*.

This species (figs. 13–16) is represented by fourteen specimens, six of which were sectioned. There is considerable variation in size, and many of them were split into three equal valves along the line of the more prominent ridges (figs. 13, 14). Like the other casts, they bear a carbonized mass at the foramen which probably represents the continuation of the inner vascular elements. They are covered with a thin carbonized film representing the sarcotesta. The only other structure present is a membranous mass about 1 mm. inside the surface, which probably represents the megaspore membrane-nucellus complex.

Trigonocarpus **serratus** sp. nov. (fig. 17)

DIAGNOSIS.—A radially symmetrical seed, elliptical in longitudinal section and circular in cross section. Sclerotesta (length 14 mm., width 7 mm.) transformed at apex into a cup-like structure, 3 mm. high, which is divided into six equal, triangular teeth, each 2 mm. long. The body is 11 mm. in length, and bears on the surface indications of three faint, equidistant ridges, between which the surface is smooth. The base is rounded, and has a carbonized mass in the foramen which represents the continuation of the inner vascular elements.

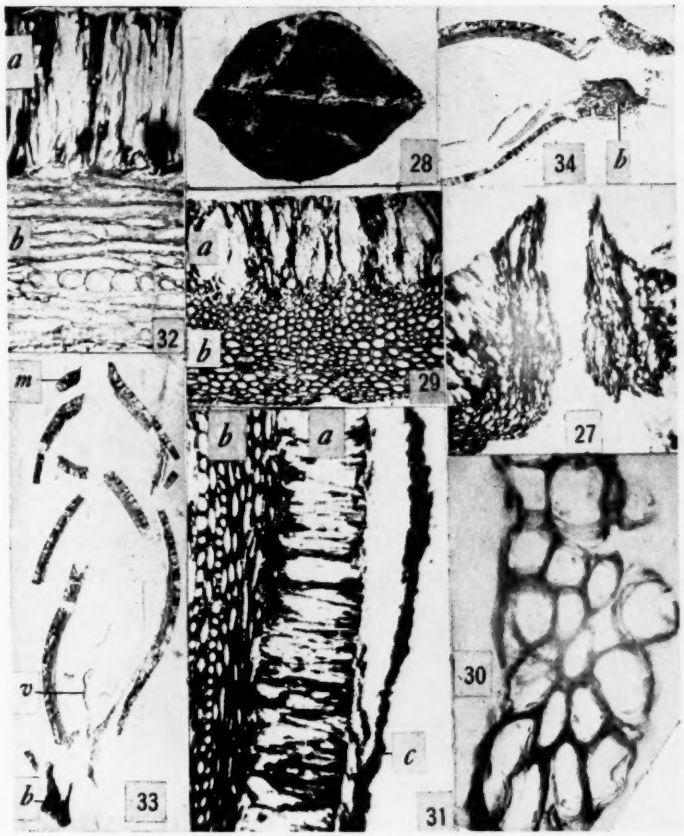
This species is represented by a single specimen, and was not sectioned. It differs from *Trigonocarpus ornatus* Newberry (**5**) in possessing only three faint ridges where that specimen bears six prominent ones, each of these being prolonged at the micropyle to form a division of the cup-like structure. In *T. serratus* the ridges, although opposite the lobes of the cup, appear to end with the main body of the seed and have no part in the formation of the cup. In *T. ornatus* the ridges are slightly constricted at the neck, and in the specimen studied there is a definite constriction between the cup-like summit and the body of the seed.

Sarcospèrmum ovale gen. et sp. nov. (figs. 27–36)

GENERIC DIAGNOSIS.—A radially symmetrical ovoid seed, circular in cross section, without ribs or sutures. Testa differentiated into three parts, sarcotesta, sclerotesta, and apparently an inner fleshy layer. Nucellus probably free to the base, and shows about 20 vascular strands in cross section.

SPECIFIC DIAGNOSIS.—Seed ovoid, 3 cm. long, with maximum width of 1.5 cm., circular in transverse section. Sarcotesta 0.04 mm. thick in its present crushed condition. Sclerotesta composed of outer palisade layer of thick walled cells 0.5 mm. in extent, and an inner layer (0.5 mm. thick) 13 to 18 elements wide of elongated fibrous cells. Sclerotesta slightly pointed at micropylar end and enlarged at basal end.

There is much similarity in the general plan of structure of the seeds of *Stephanospermum* Brongniart, *Aetheotesta* Brongniart, and *Sarcospermum*. *Sarcospermum ovale* differs from *Stephanospermum*



FIGS. 27–34.—**Sarcospermum ovale** gen. et sp. nov. Fig. 27, near median section of micropyle showing short micropylar tube. Fig. 28, surface view of seed showing part of base covered by inclosing matrix; micropylar end to right. Fig. 29, portion of cross section of seed coat: *a*, palisade cells of outer portion of sclerotesta; *b*, inner layer of sclerotesta, composed of long, isodiametric, fiber-like cells. Fig. 30, cross section of vascular bundle of nucellar vascular tissue, a mesarch bundle composed of scalariform tracheids. Fig. 31, portion of near cross section of sarcotesta and sclerotesta: *b*, fibrous layer; *c*, remains of sarcotesta, split and crushed. Fig. 32, portion of longitudinal section of sclerotesta. Fig. 33, near median longitudinal section showing badly crushed condition of tissues: *m*, micropylar region; *v*, vascular elements of nucellus; *b*, portion of base. Fig. 34, median longitudinal section of base of seed.

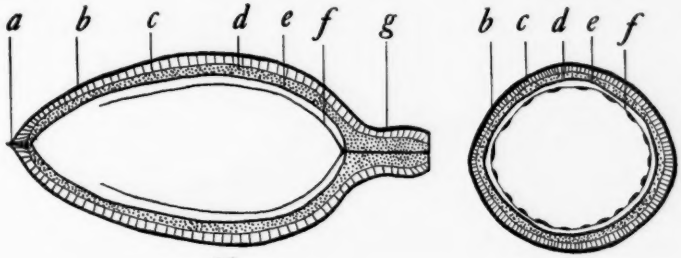
akenoides Brongniart in being of a much larger size, in lacking the long micropylar tube surrounded by a prominent crown, in having a much larger base, and in having the nucellar vascular elements divided into definite strands rather than being in a continuous sheath. *Sarcospermum ovale* differs from *Stephanospermum caryoides* Oliver (**6**) in being about twice the size of that specimen, in lacking the abbreviated crown, which has a hairy epidermis in the micropylar region, and in the structure of the nucellar vascular system as in *Stephanospermum akenoides*. In general structure *Sarcospermum ovale* approaches *Aetheotesta elliptica* Renault somewhat more closely than it does the other two species. *Sarcospermum ovale* differs from this specimen principally in having a much more enlarged base, and in possessing a sarcotesta which is of uniform thickness over the entire seed, rather than one that is much enlarged on both the basal and the micropylar ends and thin about the middle. The micropylar portion of the sarcotesta in *A. elliptica* is lacunose in nature, and the sclerotesta is prolonged into a micropylar tube. The nucellar vascular systems in *A. elliptica* and in *S. ovale* are divided into definite strands, but they appear to reach farther into the seed cavity in the latter.

This seed from Wilmington, Illinois, was so crushed and broken during the process of fossilization that it is difficult to determine accurately its former shape (fig. 33).

The sarcotesta was present over the entire seed, except at the micropylar canal, and is of uniform thickness. It was so crushed and decayed that it is impossible to determine its former thickness. This layer is split in places (fig. 31 *c*), and parts of it appear outside of a layer of calcite, about 1 mm. thick, which surrounds the seed.

The sclerotesta completely invests the seed except at the upper end where it is perforated by the micropylar canal (fig. 27). This tissue is composed of two distinct layers of cells. The outer is a single row of thick walled palisade-like cells which extend at right angles to the inner layer. These cells are about 0.5 mm. in length, and are of uniform thickness over the seed except at the micropylar and basal ends. In the region of the micropylar beak they are considerably elongated and gradually come to take a position nearly parallel to the vertical axis (figs. 27, 35 *a*). In the extreme basal

region these palisade cells are much elongated and are at an angle of about 30° to the longitudinal axis (figs. 34, 35). They are distinctly separated from the layer beneath, and are mostly filled with a carbonized, opaque substance. These palisade-like cells appear to be the outermost layer of the sclerotesta. The inner layer is composed of elongated thick walled cells which are isodiametric in cross section (figs. 29, 32). These cells extend lengthwise in the seed for the most part, but are often tortuous and twisted irregularly about one another, especially in the basal region (fig. 34). This tissue makes



FIGS. 35, 36.—Fig. 35, reconstructed median longitudinal section of seed: *a*, micropyle; *b*, remains of sarcotesta; *c*, palisade layer of sclerotesta; *d*, fibrous layer; *e*, inner flesh; *f*, vascular tissue of nucellus; *g*, large base of seed. Fig. 36, reconstructed cross section of middle of seed: *f*, nucellus showing about 20 vascular bundles.

up the greater part of the relatively large base of the seed, which is about 4 mm. long and 4 mm. thick below the body of the seed (fig. 34). The innermost layer of the sclerotesta is composed of the same elongated thick walled cells as the rest of the fibrous layer, and does not have the appearance of a definite epidermal layer.

There is no definite tissue which may be called the inner flesh, but there is a region just inside the sclerotesta which has a darker color and appears to be the debris from some tissue. This, and the fact that the sclerotesta has no finishing layer, or epidermis, would indicate that the inner flesh was present.

There is some question as to whether this seed shows a nucellus. At some distance inside the sclerotesta appears a definite membranous structure, which is much shrunken, and bears on its inner face about 20 definite vascular bundles. There are traces on the out-

side of this membranous structure of thinner walled tissue which has an amber colored material inside the cell walls. It cannot be determined definitely whether these belong to the integument or to the nucellus, but apparently they belong to the latter structure. The vascular bundles are made up of scalariform tracheids, and each bundle contains from a few of these elements to more than 25 (fig. 30). The bundles appear to be mesarch, with the smallest elements near the center of each bundle although it was not definitely determined whether these were protoxylem. The vascular system extends from the base of the seed cavity to a region in the neighborhood of the probable location of the pollen chamber. There are no vascular elements through the base of the seed, contrary to expectation, nor was a pollen chamber seen. There is no indication of any tissue inside of the nucellar vascular system.

Summary

1. A histological study was made of about 100 sandstone casts from the Lower Pennsylvanian strata of Arkansas, and one seed coat from Illinois.

2. Six species of the Trigonocarpales are described, five of them belonging to the genus *Trigonocarpus* Brongniart, and one being a new genus and species. The plants described are: *T. dawesi* Lindley and Hutton, *T. noeggerathi* Brongniart, *T.* **ouachitensis** Deevers, *T.* **costatus** Deevers, *T.* **serratus** Deevers, and **Sarcospermum ovale** Deevers.

The writer wishes to acknowledge the assistance received from various members of the Department of Botany of the University of Chicago, where the investigation was conducted.

MISSISSIPPI COLLEGE
CLINTON, MISSISSIPPI

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EMBRYO ABORTION IN THE PEACH IN RELATION TO CHEMICAL COMPOSITION AND SEASON OF FRUIT RIPENING[1]

H. B. TUKEY AND F. A. LEE

(WITH TWO FIGURES)

Introduction

That early-ripening varieties of the peach (*Prunus persica* Batsch.) produce abortive seed has been pointed out by CONNORS (3) and CHANDLER (2). TUKEY (11, 12) has shown that this characteristic appears also in early-ripening varieties of the sweet cherry (*Pr. avium* L.) and the sour cherry (*Pr. cerasus* L.). Furthermore, there is a relation between development of the pericarp and development of the embryo (12, 13, 14).

The pericarp of the peach develops in three stages (5, 6, 13). Stage I is characterized by rapid increase in size for a fairly definite period following bloom (49 to 53 days in 1934), and is of relatively uniform duration for all varieties, regardless of season of fruit ripening. In stage II development of the fruit is retarded for a variable period, depending on the variety. For a variety which ripens early (91 days from full bloom) this period is short, 5 days for Greensboro; while for a variety which ripens late (144 days from full bloom) it is long, 42 days for Chili. In stage III, known as the final swell, the fruit increases rapidly in size for a period which extends from the termination of the stage of retarded development until final ripening. The difference between an early-ripening and a late-ripening variety of peach, therefore, is largely a matter of duration of stage II.

Development of the embryo does not parallel that of the pericarp. During stage I the embryo develops slowly, so that 49 to 50 days after full bloom it is still microscopic. At the beginning of stage II, however, the embryo enters a period of rapid development which continues until it reaches maximum size for the variety. In the case of a very early-ripening variety, stage III is initiated while the

[1] Journal Paper no. 174 of the New York State Agricultural Experiment Station.

embryo is in this period of rapid development. The embryo fails to reach maximum size, the nucellus and integuments collapse, and a shriveled or abortive seed results. In the case of a late-ripening variety, the beginning of stage III does not occur until the embryo is nearly full size. Such seeds are viable, and will germinate following a period of after-ripening.

Embryos of early-ripening varieties often seem flaccid even though appearing to fill the integuments, and they may shrivel when excised and exposed. In contrast, embryos from most late-ripening varieties are firm and do not shrivel.

Such differences in texture at once suggest differences in composition. LOTT (8) has analyzed peach seed at different stages in development within the fruit and has shown it to undergo marked chemical changes. His analyses, however, did not include separation of the seed into its constituent parts, as embryo, endosperm, nucellus, and integuments.

The work reported in this paper was undertaken to determine the changes which occur in embryos during development within the seed, to compare the development and chemical composition of embryos of varieties ripening at different dates, and to throw light upon related problems of fruit, seed, and embryo development.

Material and methods

Ten varieties of peaches of different seasons of fruit ripening, extending from the early Greensboro which ripens August 3 to the late Salwey which ripens October 16, were selected for study. Fruits of these varieties were collected at or near the time of ripening, and the embryos examined and analyzed. Fruits of Greensboro, Governor Hogg, Early Rose, Triumph, and Rosebud were collected August 23, several days after the fruit was ripe. Fruits of Mamie Ross, Rochester, Lola, Elberta, and Salwey were collected when ripe. In addition, fruits of Elberta were collected during the growing season, and embryos analyzed at five periods, namely, July 10, July 21, July 31, August 25, and September 12, thus affording a comparison between the development and composition of embryos of early-ripening varieties and that of embryos of a late-ripening variety on the same date.

In preparing the material, seeds were removed from the fruits, and the integuments, remaining nucellus, and endosperm dissected away, leaving only the embryos. These were immediately weighed and divided into three samples for determination of fat, nitrogen, and sugars. The samples for fat (ether extract) were dried at 95° C. for two hours and then placed in a vacuum oven at 70° C. until they reached constant weight. The percentage of moisture was determined from these samples. The samples for sugar were placed directly in 80 per cent alcohol.

Fat was determined by the common ether extraction method; sugars by the method involving extraction by 80 per cent alcohol (1); and sucrose after inversion with invertase. The Munson and Walker method by direct weighing was used for all sugars. The determinations for reducing sugars were calculated as glucose. For nitrogen the Gunning method was used (1), excepting in some cases where the amount of sample available was limited, when the semi-micro-Kjeldahl method of UMBREIT and BOND (15) was employed.

Calculations were made on both the fresh weight and the unit basis, but since there was no appreciable difference in results, the fresh weight results alone are given.

Results with Elberta

The condition and chemical analyses of embryos of Elberta taken at different stages in development are given in table 1. Seeds examined in the first sampling, July 10, contained embryos which were approximately one-third the length of embryos in mature seeds and were increasing rapidly in size. The integuments had already reached maximum size. Such seed, when removed from the fruit and exposed in the laboratory, promptly lost moisture. Subsequently the nucellus and integuments collapsed upon the embryo, giving the appearance of abortive seed. Successively later samplings showed increasingly larger embryos, a nearer approach to complete filling of the integuments, and less flaccidity. Mature seeds of this variety contained firm embryos which completely filled the integuments.

The moisture content decreased steadily from earliest to latest samplings. Fat increased during the same period. Moisture, however, was lost more rapidly than fat was accumulated, so that the

change was not merely replacement of water by fat. Other materials were also accumulating. Nitrogen increased slowly but steadily. Reducing sugars were found only as traces in the earliest sampling and remained at a relatively low level thereafter, reaching a maximum during the period of greatest accumulation of storage materials, and then declining as the time of fruit ripening approached. Sucrose appeared in small quantities in the earliest sampling and then increased with successivelv later samplings. No starch was found.

TABLE 1

CHEMICAL ANALYSES AND STAGES OF DEVELOPMENT OF EMBRYOS OF ELBERTA PEACH AT DIFFERENT STAGES DURING SEASON.
FRESH WEIGHT BASIS

Date of sampling	Length (mm.)	Degree of filling	Condition	Percentage					
				Moisture	Ether extract (fat)	Nitrogen	Calculated protein (N × 6.25)	Sucrose	Reducing sugars as glucose
July 10..	6.5	One-third	Flaccid	93.10	0.37	0.50	3.13	0.14	Trace
July 21..	17.5	Nearly full	Flaccid	91.98	0.40	0.68	4.25	1.06	0.25
July 31..	19.0	Full	Flaccid	86.24	2.93	1.10	6.88	0.99	0.14
Aug. 25..	19.0	Full	Slightly flaccid	58.72	18.88	2.64	16.50	1.26	0.96
Sept. 12..	19.0	Full	Firm	45.63	30.67	2.60	16.25	2.32	0.23

Results with varieties ripening at different dates

The composition and characteristics of the embryos of nine varieties at the time of fruit ripening of each variety are given in table 2. In the case of the earliest-ripening variety (Greensboro) the embryos had aborted at such an early stage, were so small, and had disintegrated so badly, that sufficient material could not be secured for chemical analysis.

The moisture content declined steadily from the earliest ripening to each successively later-ripening variety. At the same time the fat increased steadily, following a linear curve, up to about 30 per cent, remaining fairly constant thereafter. Nitrogen likewise in-

creased in varieties ripening successively later, but more slowly. Sucrose appeared in the earlier varieties and remained at a nearly constant level in later-ripening varieties. Reducing sugars were not present in the embryos of the earliest-ripening varieties, were found only in traces in later varieties, reached a constant although fairly

TABLE 2

CHEMICAL ANALYSES AND STAGES OF DEVELOPMENT OF EMBRYOS OF PEACH VARIETIES RIPENING AT DIFFERENT DATES. FRESH WEIGHT BASIS

VARIETY	DATE OF FRUIT RIPENING (1936)	ABORTION (%)	DEGREE OF FILLING	CONDITION	PERCENTAGE					
					MOISTURE	ETHER EXTRACT (FAT)	NITROGEN	CALCULATED PROTEIN (N × 6.25)	SUCROSE	REDUCING SUGAR AS GLUCOSE
Greensboro	Aug. 3	100	Half	Disintegrated	.....	.....	.....	.....	.....	.....
Governor Hogg	Aug. 4	38	Two-thirds	Flaccid	85.25	1.14	.....	.....	1.70	0.0
Early Rose	Aug. 5	40	Two-thirds	Flaccid	81.24	2.40	1.34	8.38	.....	0.0
Triumph	Aug. 7	15	Five-sixths	Flaccid	76.98	4.83	.....	.....	3.93	Trace
Rosebud	Aug. 16	0	Nearly full	Flaccid	69.89	7.97	1.66	10.38	4.32	0.40
Mamie Ross	Aug. 23	4	Full	Flaccid	64.72	13.42	1.63	10.19	3.47	0.21
Rochester	Aug. 25	0	Full	Slightly flaccid	63.19	14.97	1.77	11.06	3.31	1.18
Lola	Aug. 26	2	Full	Firm	60.14	15.86	1.73	10.81	3.60	0.25
Elberta	Sept. 12	0	Full	Firm	45.63	30.67	2.60	11.25	2.32	0.23
Salwey	Oct. 16	0	Full	Firm	38.95	29.82	3.13	19.56	3.70	0.19

low level in later-ripening varieties, and then declined slightly in the latest-ripening variety. No starch was detected in any of the samples, either by microscopic examination or by treatment with diastase.

A relation appeared between decreasing amounts of moisture and increasing amounts of fat in successively later-ripening varieties. In the earliest-ripening varieties the comparative decrease of moisture was greater than the increase of fat. In varieties ripening a little later the decrease in moisture was accompanied by an equivalent

increase in fat, and in the latest-ripening varieties the still smaller amount of moisture was not accompanied by a greater amount of fat. No doubt the embryos of earlier-ripening varieties would in time have lost moisture, likewise. Such a loss was not detected because analyses were made promptly after fruit ripening.

The fact that the smaller amount of moisture in the latest-ripening variety was not accompanied by a greater amount of fat may be due to the embryos having reached maximum development considerably before the fruit was ripe. Since the sampling was done at fruit maturity, the embryo may have lost moisture during this interval.

Discussion

Growth of the embryo of the Elberta variety is plotted in figure 1, together with the moisture, fat, and nitrogen content. The graph shows clearly that the embryo reached nearly maximum size, as measured by length, before any appreciable accumulation of storage materials began. The loss of moisture and the accumulation of fat and nitrogen follow a linear curve for the most part.

Accordingly, when the embryo was making greatest increase in size the moisture content was high and the concentration of storage materials was low. As the embryo approached maximum size, accumulation of sucrose began, followed in turn by loss of moisture and accumulation of fat. Inasmuch as the nitrogen occurs largely as glucosides and proteins, the calculated protein values more nearly approximate the total quantity of nitrogenous substances actually present in the embryo than do the figures for nitrogen alone. The fat and protein together comprise 41 to 49 per cent of the fresh weight at maturity. The results are similar to those reported by FINCH (4) and by THOR and SMITH (10) for the development of the pecan kernel, and by REEVES and BEASLEY (9) for the development of the cotton embryo.

The data dealing with the other nine varieties show a definite trend and relationship between varieties. Thus in figure 2, where moisture, fat, and nitrogen content of each variety are plotted against time of fruit ripening, the resulting graph appears similar to that for the development of a single variety (Elberta) throughout the season (fig. 1).

The entire situation suggests a steady increase in growth, loss of moisture, and accumulation of storage materials in all varieties, and

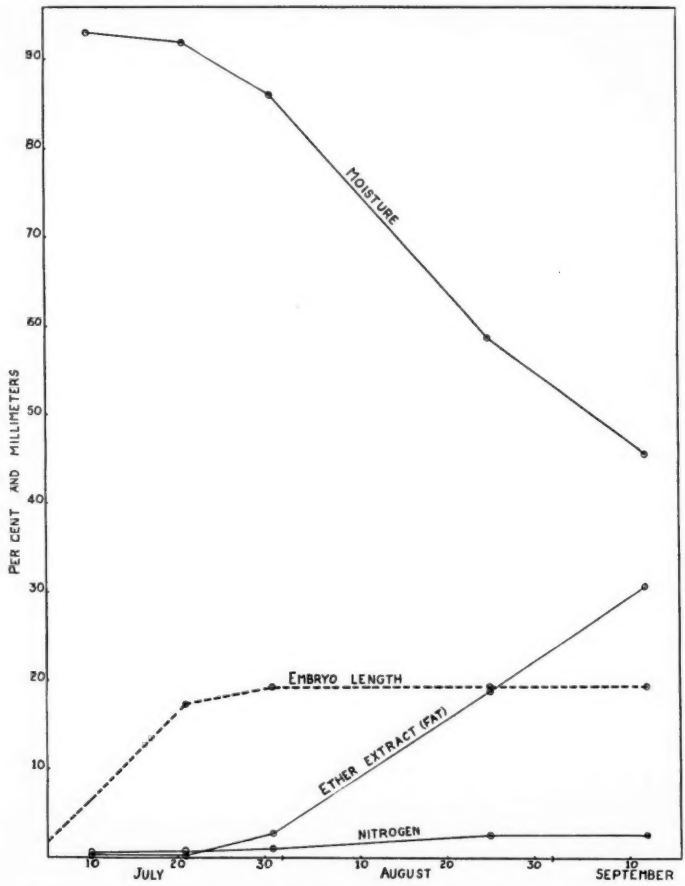

FIG. 1.—Growth of embryo of Elberta peach in relation to loss of moisture and accumulation of fat and nitrogen during development.

an abrupt check in these processes as the successive ripening dates for the different varieties are reached.

It has already been observed (**13**, **14**) that the rate and duration of

the growth periods of peach embryos are strikingly similar regardless of wide differences in season of fruit ripening. That is, during the

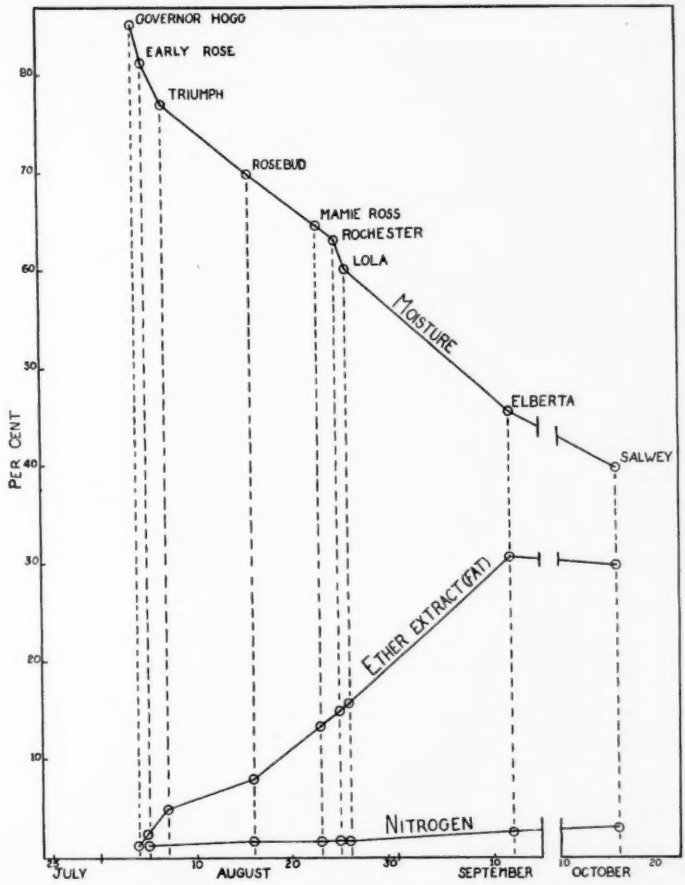

FIG. 2.—Comparison of moisture, fat, and nitrogen content of nine varieties of peaches at time of fruit ripening (*cf.* fig. 1).

season of 1934 with varieties ripening as widely apart as 91 and 144 days after full bloom, embryos of all varieties developed slowly for a period of 50 days following full bloom and then abruptly entered a

period of rapid development. A similarly striking relation is seen to exist in the chemical changes within the embryo.

The abruptness of the check in rapid accumulation of storage materials near the time of fruit ripening indicates a sharp interference with the supply of materials. The data suggest either an interruption in the vascular structure supplying the embryo or a shifting of the supply of nutrients from the embryo to some other portion of the plant. Such a shift might be thought of as being brought about by an accelerated metabolic activity in some other part of the plant, by a change in nutritional balance within the embryo, by altered enzyme activity, or by the appearance or disappearance of some growth substance. The controlling mechanism is of vital interest in connection with embryo abortion and fruit ripening in early-ripening varieties of stone fruits, and merits further study.

The relatively high moisture content of embryos of early-ripening varieties helps to explain the manner in which seeds of these varieties become shriveled, for not only may a seed shrivel because it contains an abortive embryo which never completely fills the integuments, but also because an embryo which does completely fill the integuments may later lose moisture and shrink. The relatively lower fat, nitrogen, and sucrose content of embryos of early-ripening varieties is also of interest. It will be recalled that the gross structure of the embryo is completed at a relatively early date, when accumulation of these materials and particularly of fat is low. For example, in the development of Elberta, the embryo had reached maximum size for the variety and completely filled the integuments 43 days before the fruit was ripe, yet the fat content was only 2.93 per cent as compared with 30.70 per cent at fruit ripening. In contrast, embryos of an early-ripening variety (Rosebud) which nearly filled the integuments at fruit ripening contained only 7.97 per cent fat.

LILLELAND and NEWSOME (7) describe similar results for the Elberta in California. They report that when the developing embryo is 90.6 per cent of maximum length for the variety, the fat content of the seed is only 8 per cent of what it is at maturity. In discussing the development of the cherry they say further (7) that tentative data on fat analyses of seeds of the Early Purple Guigne, Chapman,

and Mazzard indicate that the three early-ripening kinds are considerably lower in fat than the later-ripening Mazzard. They suggest that such data show that maturity of the seed of the cherry as measured by increase in fats and total solids does not take place until after the embryo has made its major length growth, and that in the early varieties this may lead to a seed low in fat and lacking viability. Yet they do not consider such seed to be abortive, and accept an embryo as abortive only "when examination reveals premature cessation of growth or evidence of disintegration *while the fruit is on the tree.*"

Using this concept, they have examined seed of two early-ripening and four later-ripening varieties of sweet cherry. Although they interpret the data to show that early-ripening cherries, as grown at Davis, California, do not have abortive embryos, yet they show that Early Purple Guigne and Chapman, the two earliest-ripening varieties of the six which they studied, produce 5.7 and 9.3 per cent abortive seed, respectively, while for three of the four later-ripening sorts (Lambert, Downer, and Lewellyn) they report 0.0 per cent abortive seed.

From the data presented by them there can be no doubt that the embryos of their Early Purple Guigne developed to larger size in California than do embryos of that variety in New York (**12**). Yet the apparent disagreement between the data in California (**7**) and those in New York would seem to be mostly one of degree and of definition. In the essentials they appear to be in entire agreement.

Using the word abortive in its meaning of checked or imperfectly developed, an embryo which fills the integuments while on the tree, but which fails to develop storage materials, is just as truly abortive as one which has failed to fill the integuments. The fact that accumulation of storage materials lags behind increase in size does not eliminate the accumulation of stored products from being a part of full functional development.

Summary

1. Embryos of ten varieties of the peach, whose several seasons of fruit ripening extend from early to late, were examined and chemically analyzed at or near the time the fruit was ripe. For compari-

son, embryos of a single late-ripening variety (Elberta) were analyzed periodically throughout the season.

2. Embryos of Elberta were flaccid and only partially filled the integuments during stage II of fruit development, so that seed became shriveled when removed from the fruit. At the time of fruit ripening, embryos were firm and completely filled the integuments.

3. Embryos of the earliest-ripening variety were abortive and disintegrated at fruit ripening. Embryos of each successively later-ripening variety were larger, firmer, and more nearly filled the integuments.

4. At fruit ripening, embryos of a late-ripening variety contained 45.03 per cent moisture, 30.67 per cent fat, 2.60 per cent nitrogen, 2.32 per cent sucrose, and 0.23 per cent reducing sugars. Embryos of early-ripening varieties showed a higher moisture content and lower content of fat, nitrogen, reducing sugars, and sucrose.

5. There is a steady increase in growth, loss of moisture, and accumulation of storage materials in embryos of all varieties, and an abrupt check in these processes as the ripening date for each successive variety is reached.

6. If the moisture, fat, and nitrogen content of the embryos of the nine varieties are plotted with reference to the time of fruit ripening, the composite graph so formed is similar to that for the development of a single variety.

7. The close similarity already observed in growth of embryos in different varieties, regardless of season of fruit ripening, extends to chemical changes within the developing embryos as well.

NEW YORK STATE AGRICULTURAL
EXPERIMENT STATION
GENEVA, NEW YORK

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HYDATHODES IN THE GENUS EQUISETUM

MARION A. JOHNSON

(WITH EIGHT FIGURES)

Introduction

BURGERSTEIN (1) has reported the occurrence of guttation in 333 genera from 115 widely distributed families. It is not so well known, however, that *Equisetum* is a convenient and suitable subject for demonstrating the secretion of water. *Equisetum scirpoides*, *E. hiemale*, *E. variegatum*, and *E. arvense*, all of which may be readily grown in the greenhouse, are satisfactory for this purpose. When well watered plants are placed under a bell jar, conspicuous drops soon appear on the leaves and sheaths. This phenomenon is most pronounced in young shoots but it may also be seen in mature ones. *E. scirpoides* has not only proved to be suitable for illustrating guttation but is an easily available source from which samples of the liquid may be collected for chemical analysis.

ROBERT (4), apparently one of the earliest to record the occurrence of guttation in *Equisetum*, mentions that *E. arvense* and *E. fluviatile* secrete water which appears in prominent drops on the leaves. Later VOLKENS (9), in a paper devoted to the secretion of water in the higher plants, states that water drops are formed on the leaf and sheath of *E. arvense* and *E. limosum* and that they are secreted by stomata located on the inner face of the leaf sheath. Since then SCHAFFNER (6) has reported guttation for *E. praealtum*, *E. hiemale*, and *E. variegatum*. So far as the writer is aware, no adequate descriptions or figures of the hydathodes in *Equisetum* have appeared in the literature, except for the brief reference made to them in an earlier paper by JOHNSON (3) on *E. scripoides*. The species shown on the following page, including practically all of the living species of the genus, and arranged according to SCHAFFNER'S (5) classification, have been studied.

The material examined consisted of leaves and leaf sheaths from both herbarium and living specimens. These were cleared for sev-

eral days in a saturated solution of chloral hydrate and examined *in toto;* in addition, thin sections were made by the paraffin method and stained in safranin and fast green.

EQUISETA PRIMITIVA
E. xylochaetum Mett.[1]
E. giganteum L.[1,4]

EQUISETUM HIBERNA
E. ramosissimum Desf.[1]
E. debile Roxb.[1]
E. laevigatum A.Br.
E. praealtum Raf.
E. moorei Newm.[1]
E. hiemale L.
var. *californicum* Milde.[2]

EQUISETA AMBIGUA
E. kansanum Schaffn.[3]
E. funstoni A.A.Eat.[2]

EQUISETA PUSILLA
E. nelsoni (A.A.Eat.) Schaffn.[1]
E. trachyodon A.Br.[1]
E. variegatum Schleich[1]
E. scirpoides Michx.

EQUISETA AESTIVALIA
E. fluviatile L.
E. palustre L.
E. bogotense H.B.K.[1]
E. diffusum D.Don.[1]

EQUISETUM HETEROPHYADICA
E. silvaticum L.
E. pratense Ehrh.
E. telmateia Ehrh.
E. arvense L.

Observations

Hydathodes were found in each of the twenty-two species examined and probably occur in every species in the genus. They are confined to a restricted area on the adaxial surface of the leaves or sheath, or on both, depending upon the species. This region (figs. 1–3), which is considerably lighter in color than the adjacent chlorophyllose tissue and which is always intimately associated with the adaxial face of the foliar vein, constitutes a single hydathode. In *E. variegatum*, *E. palustre*, *E. ramosissimum*, and *E. giganteum*, where the leaves are discolored, it can readily be recognized by the absence

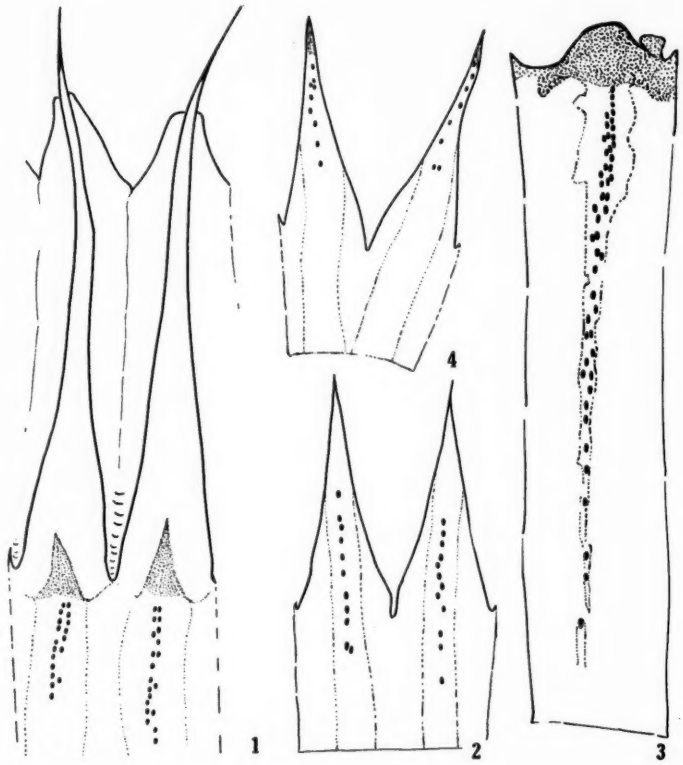
[1] Obtained from the New York Botanical Garden through the courtesy of the Director.

[2] Supplied by Dr. A. W. HAUPT of the University of California at Los Angeles.

[3] Furnished by Dr. ELDA R. WALKER of the University of Nebraska.

[4] Collected in Mexico by Dr. C. J. CHAMBERLAIN of the University of Chicago and in Jamaica by Dr. M. A. CHRYSLER of Rutgers University.

of coloring matter in its epidermal cells. The hydathodes are much longer than broad and are studded with pores resembling stomata in general appearance but differing from them in their position above a



FIGS. 1–4.—Fig. 1, *E. hiemale* var. *californicum:* leaves and sheath showing position of water stomata in hydathode. Fig. 2, *E. arvense:* same from main axis of aerial shoot showing position of water stomata in hydathode. Fig. 3, *E. giganteum:* sheath from main axis showing position of water pores in hydathode. Fig. 4, *E. arvense:* leaves and shoot from ultimate lateral branch of aerial shoot showing position of water pores in hydathode. ×16.

vein. The average length for the hydathodes throughout the genus is about 1 mm. They are, however, longer in the large leaves; for instance in *E. giganteum* they are often 5 mm. long (fig. 3), and may

extend for a distance of 15–20 mm. on the leaves of the fertile shoot of *E. telmateia*.

Studying the distribution of the hydathodes throughout the genus, it was found that they either extend well up into the leaves (figs. 2, 4) or are confined to the sheath and leaf bases (figs. 1, 3). The former condition is seen in the Equiseta Aestivalia and Equiseta Heterophyadica, where the leaves are persistent, contain considerable photosynthetic tissue, and do not dry out at maturity; while the latter occurs in the Equiseta Primitiva, Equiseta Hiberna, and Equiseta Ambigua. The species in the last mentioned three sections have deciduous leaves and thus the basal position of the hydathodes permits their retention when the leaves are shed. This arrangement may be of value to the species in question, yet the position of the hydathodes appears to be correlated with the thickness of the leaves rather than with their deciduous nature. The Equiseta Pusilla bear out this hypothesis, for here the leaves are not only characterized by their extreme thinness and early desiccation, as in the Equiseta Primitiva, Hiberna, and Ambigua, but also by being persistent; and it is significant that the hydathodes are confined to the sheath and leaf bases just as they are in the species with deciduous leaves.

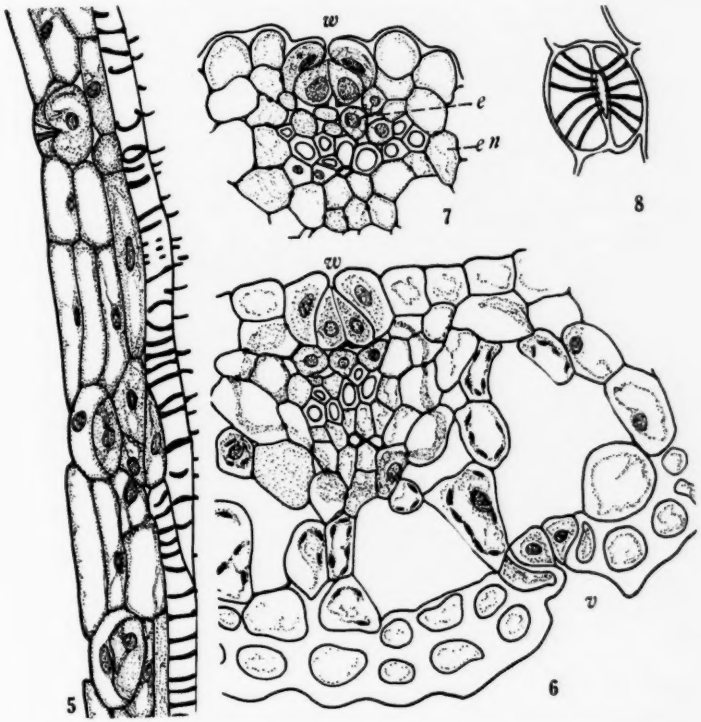
A detailed study of the distribution of the hydathodes in *E. arvense* shows them to be present on the leaves of both the sterile and fertile shoots, and of the rhizome as well. One notable exception is the case of the ocreole or first sheath of the branches, where they are absent. This is not surprising in view of the reduced condition of the ocreole, for it is only a few cells in thickness and may be devoid of vascular tissue. Sometimes veins are differentiated, however, but even so hydathodes are not developed. The hydathodes on the rhizomes are definitely restricted to the sheath and base of the leaves and measure from 1 to 1.5 mm. in length, while those from near the apex of the sterile shoots are confined to the leaf tips and are reduced in length to about 0.5 mm. The gradual decrease in length and the restriction of the hydathodes to the distal end of the leaves parallel the reduction in leaf size seen in passing from the rhizome to the ultimate nodes of the sterile branches. This relationship between leaf size and length of hydathodes is clearly shown in the large conspicuous leaves of the fertile shoot of *E. telmateia*, where they may be 15 to 20 mm. in length.

Evidence is not at hand to show whether the hydathodes from the rhizomes are functional. They are a constant feature in *E. arvense* and *E. silvaticum*, however, but even in these species those more than three nodes below the soil level have imperfectly developed water pores, so that there is some doubt as to their ability to secrete water. In *E. scirpoides* and *E. praealtum*, hydathodes were not observed from the rhizomes, and unfortunately suitable material was not available for further study concerning this point in the remaining species.

Longitudinal and transverse sections through the leaves (figs. 5–7) show that in *Equisetum* the hydathodes are simple in structure. At most they consist of a few layers of epithem tissue derived from the pericycle on the adaxial face of the xylem, an endodermis which is extended until it joins the epidermis, and a varying number of water pores opening upon the adaxial surface of the leaf or sheath throughout the length of the hydathode. Occasionally the epithem tissue is so poorly developed that a lignified cell of the xylem lies exposed beneath a water pore. Dense cytoplasm, large nuclei, few if any chloroplasts, and thin cellulose walls characterize the epithem cells. They are also considerably shorter than the cells which lie in a corresponding position below the hydathode and their arrangement allows for small intercellular spaces in the vicinity of the water pores (figs. 5–7).

It has been assumed thus far that the areas described as hydathodes really do function in the secretion of water, and there is evidence supporting this assumption. When the hydathodes are conspicuously located at the tip or edge of the leaves, as is the case in the Crassulaceae, it is relatively easy to trace the drops of water from guttation to their source. In *Equisetum*, however, where the leaves are small and closely appressed to the stem this is not so readily accomplished. Here the water collects above the node between the leaf and stem. Thus there are two natural openings from which it might have escaped, the stomata on the stem immediately above the node and the pores on the adaxial face of the leaf and sheath. Considering the first possibility, it can be stated with certainty that the stomata are not underlaid with secretory tissue, that they are not in close proximity to the vascular bundles, and that they are

separated from the vascular tissue by a well developed endodermis. These stomata are not adapted for guttation and it is extremely doubtful whether they function in this capacity. On the other hand, the pores in the hydathodes are located above the veins, are in direct



FIGS. 5–8.—Fig. 5, *E. arvense:* structure of hydathode in longitudinal section. Fig. 6, *E. kansanum:* hydathode and ventilating stoma in transverse section; note that endodermis joins epidermis (*w*, water pore; *v*, ventilating stoma). Fig. 7, *E. arvense:* hydathode in transverse section (*w*, water stoma; *e*, epithem tissue; *en*, endodermis). Fig. 8, *E. palustre:* surface view of guard cells of a water stoma. ×375.

communication with a distinct epithem tissue which in turn is in contact with the xylem, and the whole unit is surrounded by an endodermis. The entire arrangement is not only well suited for the

secretion of water but would prevent the adjoining mesophyll tissue from becoming flooded. These facts are considered as sufficient reason for regarding the pores associated with the veins, and not the ventilating stomata, as the avenues through which the water is secreted.

The number of water stomata in a hydathode varies from three to four in the small branches of *E. silvaticum*, where the leaves are much reduced, to as many as fifty in *E. giganteum*, and the number may be even higher in the large leaves on the fertile shoot of *E. telmateia*. A comparison of the water stomata with the ventilating stomata shows them to be similar in general plan but with the former generally somewhat smaller in size, as seen in surface view. Both are derived from epidermal initials cut by two slightly oblique radial walls into three cells whose long axes parallel that of the leaf. The angle at which the walls are formed makes the central cell broadest on its inner tangential face while the flanking cells are broadest on the outer tangential faces. The central cell, upon division, gives rise to the guard cells bearing radial markings; these at maturity are overgrown by the flanking or accessory cells. Neither the accessory nor the guard cells are displaced as much as are those of the ventilating stomata, and in cross section they appear more nearly at right angles to the epidermis. There is, however, considerable variation in respect to this point (figs. 6, 7). The chief differences between the two types of stomata are to be seen in their contents and in the thickness of their cell walls. In no instance could chloroplasts be found in the guard cells of the water stomata; this point was verified from both living and stained preparations. The walls of the guard cells of the water stomata are much thinner than those of the ventilating stomata and are more nearly uniform in thickness, except for a slight thickening on the inner tangential face (figs. 6, 7).

Continuing a comparison of the water pores and ventilating stomata, it will be recalled that in the Equiseta Aestivalia and Heterophyadica the stomata are not sunken below the surface of the epidermis as they are in the remaining four sections of the genus. The water pores, however, are flush with the surface or are very slightly sunken throughout the genus (figs. 5–7). This feature is remarkably constant, regardless of whether the species are mesophytes or xerophytes, and is to be added to the list of characteristics showing little

variation in this old and stable genus. The position of the water pores on the upper surface of the appressed leaves offers considerable protection against desiccation, and may in part be responsible for the failure to develop the sunken habit in the xerophytic species.

The absence of chloroplasts and the uniform thickness of the radial walls of the guard cells raise the question as to whether it is possible for the water stomata to be opened or closed to any great extent. Examination of material preserved in formalin-acetic-alcohol generally shows the guard cells to be open (fig. 8), but in living material they appear to be closed. This point was investigated with the aid of a Leitz Ultrapak. Leaves from young stem tips of *E. praealtum* were removed while actively secreting water and mounted in water at once; some of the guard cells were slightly open but for the most part they were entirely closed.

Discussion

So far as the writer is aware, the epithem type of hydathode has always been described as being intimately associated with the free tip of tracheids, representing either the distal end of a foliar veinlet or modifications from the nodal points of the vascular network. The former condition may readily be seen in *Primula sinensis* Lindl., where the veins of the leaf teeth abut directly upon the epithem tissue, and as HABERLANDT (2) has clearly shown, some of the tracheids may even project into the intercellular spaces. The second type is illustrated by the prominent hydathodes in *Crassula quadrifida* Baker, which are supplied by tracheids arising from the tip of the veins and spreading out in the form of a fan before entering the epithem. In *Equisetum* the hydathode differs from either of these types; here the epithem is confined to the face of the vein and does not extend to its tip. The tracheids are not displaced from their course but continue undisturbed throughout and even beyond the length of the hydathode. This places the epithem cells and their intercellular spaces in direct contact with the long axis of the tracheids, and judging from the amount of water secreted, is an efficient arrangement. This type of epithem hydathode is apparently unique.

Phylogenetically water stomata are regarded as having originated from ventilating stomata. Whether this could apply in *Equisetum* was a question until stomata were found on the adaxial face of the

leaves in *E. telmateia* and *E. diffusum*. Those of the former are confined strictly to the fertile shoots, where they extend from the base of the sheath to within 15–20 mm. of the leaf tips. The aerial shoots in *E. diffusum* are not separated into sterile and fertile shoots, but instead the main axis is terminated by a cone, with the lower nodes producing sterile branches while the upper ones have none. Only the leaves from the upper nodes, and especially from the final node nearest the cone, have stomata on the adaxial face. It is significant that in each of these species adaxial stomata are associated with reproductive structures which in general are noted for their conservative nature. The discovery of these stomata in *E. telmateia* and *E. diffusum* is of considerable importance, in that it may be taken to indicate that *Equisetum* or its ancestors once had stomata on the upper surface of their leaves and that from them the water stomata of the hydathodes have been derived.

The wisdom of searching the fossil record of *Equisetum* for evidence in support of this hypothesis is obvious, for as SCOTT (**7**) has remarked, "In fact, we may safely say that any adequate knowledge of the Equisetales must be derived to a much greater extent from the study of the extinct forms than from that of the few surviving representatives." An examination of the Paleozoic Calamites whose equisetaceous affinities are undoubted shows stomata on the adaxial face of the leaves. As in *Equisetum*, the foliage was borne in verticils but the free tips were often more extensively developed, as is to be seen to good advantage in the linear, lanceolate, or spatulate leaves of *Annularia*. Unfortunately the internal structure of the foliage of *Annularia* is unknown. In *Asterophyllites* the leaves were linear or lanceolate and resembled those of *Equisetum*, except that they were not united into a sheath at the base. The anatomy of the *Asterophyllites* type of leaf has been investigated by THOMAS (**8**), who describes each leaf as being supplied with a vascular bundle which was surrounded on all sides by photosynthetic tissue. In the tip of the leaves fibers instead of parenchyma occurred above the veins. Unlike *Equisetum*, the stomata were restricted to the upper surface of the leaves, where they occurred in rows but with a tendency to be absent above the veins. No mention is made of structures which might be interpreted as hydathodes. Negative evidence, however, does not preclude the possibility of their occurrence among the Cala-

mites, especially when it is realized that the leaf structure is known for relatively few species of this great group of plants.

Taking the Calamite leaf as the general ancestral type from which the *Equisetum* leaf was derived, the origin of the hydathodes may be visualized as having occurred in the following manner. The leaves were reduced in size and the photosynthetic tissue was limited to the abaxial face of the leaf, while the vein approached the adaxial face. These changes were accompanied by a restriction of the stomata to the thicker parts of the leaf. Thus those on the adaxial face were confined to the region above the foliar vein and new ones were initiated on the abaxial face in connection with the photosynthetic tissue, as is seen in *Equisetum* today. Eventually in the leaf tips the vein touched the epidermis and the endodermis became joined to the epidermis by a Casparian strip, thereby isolating the adaxial stomata from the remainder of the leaf. A slight proliferation of the pericycle, together with the loss of the chloroplasts in the guard cells, would produce the epithem hydathode of *Equisetum*.

These stages are practically duplicated in the leaves of the fertile shoot of *Equisetum telmateia*. The adaxial face is supplied with several rows of stomata at the base, while at the tip where the leaf is much thinner they are generally reduced to a single row lateral to each side of the vein. The endodermis of the vein is continuous with the epidermis throughout the length of the hydathode, but below this point there are stomata which are not connected with the vein. In all probability these represent ventilating stomata that have been isolated above the vein during the evolutionary process.

The characteristics exhibited by the hydathode are apparently of no value in determining the degree of relationship between the species of *Equisetum*. Their structure is extremely simple and remarkably uniform throughout the genus. The most noticeable variations are in their position and in the number of water stomata. The former, however, depends upon the thickness of the leaves while the latter is correlated with leaf size regardless of the species.

Summary

1. Hydathodes of the epithem type are reported for twenty-two species of *Equisetum*.

2. The hydathodes are confined to the adaxial face of the leaf and

sheath. They extend along the foliar vein but are not associated with the free tips of the vascular elements.

3. The epithem is derived from the pericycle of the vein and is incompletely developed.

4. Pores of the water stomata type connect the hydathodes with the surface of the leaf. Their number varies with the size of the hydathodes, which in turn is related to leaf size.

5. In species with very thin or deciduous leaves the hydathodes are borne on the sheath or leaf base, while in those with persistent and relatively thick leaves they are borne on the leaf and often near the tip.

6. Throughout the genus the water stomata are on a level with the surface of the epidermis.

7. Phylogenetically the water stomata appear to be derived from ventilating stomata which have been isolated above the veins on the adaxial surface of the leaf and sheath. The presence of ventilating stomata on the adaxial face of the foliar members in *E. telmateia*, *E. diffusum*, and in certain of the Calamarieae supports this hypothesis.

BUREAU OF BIOLOGICAL RESEARCH
RUTGERS UNIVERSITY
NEW BRUNSWICK, NEW JERSEY

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FORMS OF CRATAEGUS CRUS-GALLI

H. W. RICKETT

The Crus-Galli are the largest group of *Crataegi* in Missouri, more than forty species having been described. As usual in this genus, these numerous species are distinguished by only small differences, often in such variable characters as shape and size of leaves. Most of them were described by SARGENT,[1] who later[2] was led by observations of PALMER to merge several of the supposed species. The question arises whether such an effort to reduce the multitude of named species to a more usable number may not conceal real racial differences and so obscure rather than help to elucidate the *Crataegus* problem. In a previous paper (*l.c.*) I have shown that proper methods of study reveal constant differences, not apparent to ordinary observation, between local races of *C. pruinosa* (Wendl.) Koch. A study of local forms of the Crus-Galli has yielded similar results.

In this complex group, which intergrades with the Punctatae and the Virides, can be distinguished a plexus of forms which differ only slightly among themselves and all of which are evidently closely related to *C. crus-galli* L. This species, interpreted as including a large number of such local races, may be briefly described as follows.

Shrubs, with long straight spines. Leaves coriaceous, lustrous, usually glabrous, from obtuse or rounded to acute, from obovate or spatulate to elliptic, 30–60 mm. long, about half as wide, on very short petioles (4–8 mm.). Leaves on non-flowering branches often larger and more acute, sometimes lobed, but in size and shape varying greatly from branch to branch and from year to year. Cymes of 10–20 flowers, usually glabrous; bracts deciduous before anthesis. Sepals linear, entire, or more or less gland-serrate. Stamens usually about 10 or (less often) 20. Anthers cream colored or pink. Styles 1–3. Fruit usually oblong, 8–14 mm. in diameter, more or less pruinose. Nutlets 1–3, 6–8 mm. long, usually strongly ridged dorsally; many lacking seeds.

[1] For references, see previous paper in BOT. GAZ. **97**:780–793. 1936.

[2] Jour. Arn. Arb. **6**:1. 1925.

Three forms of this species may be seen near Columbia, Missouri, differing only in number of stamens, color of anthers, and other less evident characters. Individual plants were marked and studied in successive years. Statistical studies of the numbers of stamens and styles have been attempted, but remain incomplete through a certain fatality which pursues marked trees; many of which have been felled or burned or otherwise killed, while others have failed to bloom. It seems probable, however, that the different numbers of stamens and of styles which characterize these forms are constantly associated with different colors of anthers. It is interesting that the few counts so far completed show a mean number of styles per flower in one form of 1.3; in another of 1.9; and these figures reappear in counts of the numbers of nutlets per fruit. Such analysis reveals a real difference between races which would otherwise be classed together as having "styles 1–2." Such characters are more reliable than many ordinarily used. Those of fruits, for instance, are in general so affected by the vagaries of climate and by the attacks of parasites as to be almost useless; in the forms under consideration the color of fruits varies from year to year on one plant from brick-red or bright cerise to dull crimson.

A series of measurements of spines yields a significant difference between plants classed in different groups according to their floral characters. This is shown in table 1. For purposes of comparison measurements are included also of spines of *C. danielsii* Palmer (see later) and of several of the Punctatae of this region. Measurements were made in the field on 25 spines per plant. Other measurements were made on herbarium specimens, the numbers varying from 20 to 50 per plant; these naturally are apt to represent flowering or fruiting branches more than the others, and it is therefore not surprising that the average of measurements made in the herbarium does not always coincide with that of measurements made in the field on the same plant. Such differences are certainly not significant; differences of similar magnitude between individual plants of the same group are likewise probably without meaning; but there is no question that one group is constantly characterized by shorter spines. As evidence of the reliability of the difference, it is worth recording that the classification by length of spines only of an un-

labeled plant was later found to be correct when floral characters were studied.

These data are interesting chiefly as they confirm those obtained from *C. pruinosa;* as in that species, the distinguishable characters

TABLE 1

LENGTH OF SPINES (IN MM.) IN LOCAL RACES OF CRATAEGUS

CRUS-GALLI		
Anthers cream Stamens 7–10 Styles 1–2 (1.3)	Anthers pink Stamens 15–20 Styles 1–2	Anthers pink Stamens 7–10 Styles 1–2 (1.9)
Measured in the field		
(47)* 51.5±1.10 (48) 51.3±1.34 (37) 43.3±0.71 (70) 43.9±0.90	(65a) 48.1±0.10 (69) 43.1±0.92	(71) 30.0±0.97 (60) 30.3±0.81 (46) 27.1±0.99
Measured in the herbarium		
(47) 42.5±0.77 (48) 39.9±1.54 (37) 43.2±0.91 (70) 47.2±0.73 (44) 53.1±1.70 (41a) 51.9±1.37	(65a) 40.1±1.05 (69) 40.6±0.87	(36) 30.7±1.30 (60) 24.9±1.21 (46) 28.9±0.52
C. DANIELSII	PUNCTATAE	
(35) 33.5±0.76 (8) 36.9±0.61 (8)† 35.3±0.97 (19a) 33.1±0.84	(24) 49.3±1.20 (25) 45.9±0.80 (65) 43.1±1.41	(16) 38.5±1.06 (19) 37.9±1.09 (20) 38.7±0.79

* Numbers in parentheses are those attached to individual plants for the purpose of recognition.

† Measurements made on this plant in two seasons.

are not combined at random in what CLAUSEN has called a "hybrid swarm," but are associated in a smaller number of constant combinations. The plants characterized by each of these combinations are supposedly related and capable of formal recognition. It is probable

that hybridization was involved in the origin of the numerous races of *C. crus-galli*, and probable also that their constancy and geographic restriction are results of apomictic descent from their hybrid ancestors.

It is unfortunately impossible to know whether or not these forms are identical with any of those previously described; such identification must await further studies of this kind. The form with cream colored anthers resembles *C. arduennae*, which has been identified (as already mentioned) with *C. albanthera*, *C. candens*, and *C. ferox*. The short-spined form with pink anthers and about 10 stamens resembles *C. hamata* and *C. infesta*. *C. strongylophylla* and *C. permera* also are similar except in having usually more styles. All of these forms seem to have more glandular sepals than the plants of this vicinity. The form with more numerous stamens seems to be nearly unique in this respect among pink-anthered forms of *C. crus-galli*.

Through the kind cooperation of J. M. GREENMAN, Curator of the Herbarium of the Missouri Botanical Garden, and of E. J. PALMER, of the Arnold Arboretum, I have been able to study the collections upon which SARGENT based his descriptions of the Crus-Galli of Missouri. It is difficult to interpret any particular specimens as types, but it is easy to find the specimens cited in the descriptions; and these are very uniform and closely resemble specimens from the same localities collected subsequently. Such characters as can be recognized (for instance, pubescence and margination of sepals) seem to provide constant means of differentiation between these "species." Even discounting conclusions based upon herbarium material and upon SARGENT'S detailed descriptions, the results of the present study make it probable that at least most of these named races are really distinct.

It is with hesitation that I assign names to these forms. Their existence must be recognized and their characters catalogued, if the species which they compose is to be understood. But their number is so vast and their distribution so restricted that a less elaborate system is needed for their designation; letters or numbers, perhaps. Until definite action by botanists provides for such a situation, however, it is necessary to use the system now recognized. I have discussed elsewhere my reasons for regarding these races of *Crataegus* as

forms and the complex of forms as a species. I am here concerned only with naming the forms which I have carefully studied in the field; reduction of the multitude of already described "species" must await more exact characterization of them.

Crataegus crus-galli L. **vulgaris** f. nov. Folia cymique glabra. Spinae 40–50 mm. Stamina ca. 9, antheris eburnis. Styli 1–2 (1.3). Fructus lati 10–14 mm. Nuculae longae 6–7 mm. *Rickett* no. 46, May 5, 1931 (Herb. Univ. Mo. 8959).

Crataegus crus-galli L. **staminea** f. nov. Folia cymique glabra. Spinae 40–50 mm., saepe recurvatae. Stamina ca. 15–20, antheris roseis. Styli 1–2. Fructus lati 11–12 mm. Nuculae longae 6–7 mm. *Rickett* no. 750, May 21, 1935 (Herb. Univ. Mo. 17229).

Crataegus crus-galli L. **spinulosa** f. nov. Folia cymique glabra. Spinae 25–30 mm. Stamina ca. 10, antheris roseis. Styli 1–2 (1.9). Fructus lati 10–12 mm. Nuculae longae 6–8 mm. *Rickett* no. 47, May 20, 1931 (Herb. Univ. Mo. 8964).

C. danielsii Palmer also is evidently related to the preceding forms. It is locally very abundant, on rocky hills, in old pastures, and in vacant ground in Columbia. It is a handsome shrubby tree, usually loaded with blossom or fruit. The leaves are coriaceous and shining as in *C. crus-galli*, but more often acute and frequently lobed. Spines average about 35 mm. The cymes have a thin deciduous pubescence; each bears 15–20 flowers. Bracts may persist until anthesis. Sepals are regularly gland-serrate. The 7–15 stamens have pink anthers; there are 2–5 styles, averaging close to 3. The fruits are bright crimson, 11–15 mm. in diameter, conspicuously pruinose. The nutlets bear prominent dorsal ridges, and are 6–7 mm. long.

In some of these characters *C. danielsii* approaches the Punctatae, which are distinguished from the Crus-Galli chiefly by their thinner, more acute, usually pubescent leaves with straighter, more impressed veins; pubescent cymes; more numerous styles and nutlets; more persistent bracts. PALMER suggests that it may have originated as a hybrid between the two groups. Among the Crus-Galli of Missouri many of SARGENT'S species occupy a similarly intermediate position. *C. parciflora*, *C. lawrencensis*, *C. leptophylla*, and *C. tenuispina* seem (as far as can be judged from descriptions and from herbarium specimens, with the reservations noted) to be distinguished by more nu-

merous styles and larger leaves, more often elliptic in shape. Others, such as *C. discolor*, *C. pilifera*, and *C. vallicola*, show a tendency to pubescence besides. Still others have smaller, decidedly more elliptic acute leaves, with various combinations of the other characters just mentioned; examples are *C. tantula*, *C. paradoxa*, *C. intermixta*. A fourth group seems to be marked by much broader leaves: *C. rotunda*,

TABLE 2

LEAF INDEX $\left(\frac{\text{LENGTH}}{\text{WIDTH}}\right)$ IN LOCAL RACES OF CRATAEGUS

C. CRUS-GALLI		PUNCTATAE	
(47)*	2.3±0.06	(24)	1.9±0.02
(47)†	2.7±0.05	(25)	1.8±0.03
(48)	2.2±0.04	(65)	1.6±0.03
(37)	2.0±0.03	(16)	1.8±0.03
(70)	2.2±0.08	(19)	2.1±0.04
(69)	2.4±0.06	(20)	1.6±0.03
(65a)	2.1±0.04		
(71)	2.0±0.02	C. DANIELSII	
(60)	2.2±0.01		
(46)	2.3±0.05	(8)	2.1±0.03
		(8)†	2.1±0.05
C. albanthera	2.0	(35)	2.2±0.04
C. arduennae	2.0	(19a)	2.1±0.04
C. candens	2.0		
		C. intermixta	1.7
C. parciflora	1.8	C. paradoxa	1.8
C. pilifera	2.1	C. tantula	1.7
C. lawrencensis	1.7		
C. vallicola	1.7	C. jasperensis	1.4
C. discolor	1.7	C. palmeri	1.3
		C. rotunda	1.3
		C. rubrifolia	1.7

* Numbers in parentheses are those attached to individual plants for the purpose of recognition.

† Same plant measured in different years.

C. jasperensis, *C. palmeri*, *C. rubrifolia*. Whether such apparent differences in size and shape of leaves represent real genetic characters can be determined only by the development of adequate methods of measuring such variants. In spite of great variation even on one plant, it seems that an index of shape of leaves obtained by dividing length by width is a fairly constant figure in related plants. Table 2 presents such data from several plants of the local forms of *C. crus-galli*, of *C. danielsii*, and of a few of the Punctatae of the region. The

measurements were made in the field on 25 leaves per plant. Included in the table also are similar figures computed less accurately from herbarium specimens of some of SARGENT'S species.

A further character which may be of some value in tracing relationships is time of anthesis. Near Columbia the Punctatae and *C. danielsii* flower during the first week in May, *C. crus-galli* about two weeks later. In general there is a tendency through the entire group for the more characteristic forms of *C. crus-galli* to be the latest to flower; there are, however, so many exceptions that no reliance can be placed on this single character alone.

Even from such imperfect and incomplete observations as those presented in the preceding discussion, it is evident that the species named have many of the characters of *C. crus-galli*, of the Punctatae, and perhaps of the Viridis, in various combinations. The effect is that of a "hybrid swarm" of races rather than of individuals, each race restricted in range. No particular type seems to be selected by any environment; except for those with nearly round leaves, which inhabit the southwestern counties, all types seem to occur indiscriminately in all parts of the state. In such a situation it is obviously impossible to designate certain forms as distinct species while others are not so recognized. At the same time, such types as *C. danielsii* differ more from the characteristic forms of *C. crus-galli* than do the latter among themselves, and therefore must be differently ranked. The most convenient method perhaps is to group all these intermediate types into a number of varieties associated with the species which they most nearly resemble. *C. danielsii*, judging from the scanty pubescence (which is sometimes lacking), from the proportions and texture of leaves, and from the numbers of floral parts, seems rather more closely related to *C. crus-galli* than to the Punctatae. I propose therefore the following nomenclatural change:

Crataegus crus-galli L. var. **danielsii** (Palmer) comb. nov. (*C. danielsii* Palmer in Jour. Arn. Arb. **16**:355–357. 1935).

An example of the dangers of reliance on herbarium specimens and of isolated observations is *C. danielsii* f. *glabra* Palmer (*l.c.*). This was based on a specimen in the herbarium of the University of Missouri. Unfortunately the group of plants from which the specimen came has been destroyed, and search has discovered no others

near by. But the real status of this "form" is shown by examination of various collections of var. *danielsii*. One of the marked plants (no. 8), as represented in the herbarium, had cymes scantily villose in 1931, glabrous in 1935; another plant (no. 35) likewise yielded pubescent specimens in 1931, while in 1936 careful examination in the field showed that some cymes were distinctly pubescent, others glabrous. The pubescence of leaves parallels that of cymes. The supposed glabrous form was doubtless one of the occasional glabrous branches of a variety characterized by a variable and deciduous pubescence.

DEPARTMENT OF BOTANY
UNIVERSITY OF MISSOURI

BRIEFER ARTICLE

FURTHER OBSERVATIONS ON GONYOSTOMUM SEMEN

Gonyostomum semen (Ehrenb.) Diesing, a chloromonad flagellate, the general morphology of which has recently been described (3), was found to persist in the plankton of Cedar Swamp near Woods Hole, Massachusetts, during the summers of 1935 and 1936. In August 1936 it was discovered in even greater abundance in Iron Pond, a waterhole beside the railroad half a mile west of the swamp. Iron Pond is not situated in a sphagnum bog as is Cedar Swamp; but its plankton, like that of the latter, is composed mainly of flagellates.

During the summer of 1935, material from Cedar Swamp was fixed in Zenker's and Schaudinn's fluids (the latter containing 5 per cent glacial acetic acid by volume) prepared according to formulae in Lee's *Microtomist's Vade-Mecum* (1928), was impregnated with paraffin by the usual methods, sectioned at a thickness of 6 μ, stained with Heidenhain's iron-alum haematoxylin, and mounted in balsam. Certain morphological features not known at the time of writing the 1935 paper were brought to light. The flagella, often preserved *in toto* by fixing in Zenker's fluid, were found to be of approximately equal length; their basal portions extend through the opening of the triangular cavity and are inserted in the cell membrane in the center of the base of the cavity. No blepharoplasts could be identified at the points of insertion of the flagella, but distinctly chromatic threadlike structures were seen extending from these points of insertion to the anterior end of the nucleus, where no bodies could be recognized as centrosomes. The nucleus, as studied in the stained sections, is an oval body apparently surrounded by a nuclear membrane. Inside this apparent membrane is a matrix of small closely packed chromatic granules of rather regular size which stain conspicuously with Feulgen nucleal reagents. Scattered at random through the nuclear matrix are from one to ten larger chromatic bodies (endosomes?) measuring 1–2.5 μ in diameter. Figure 1 gives a diagrammatic representation of the nucleus and of the kinetic apparatus as we have observed them. The picture of the resting nucleus is much the same as that described by FOTT (4) in *Vacuolaria viridis* (Dang.) Senn, but rather different from that indicated in the original description and figures of another chloromonad,

Reckertia sagittifera Conrad (2). The kinetic apparatus of *G. semen* as here represented is far less complex than that described by FOTT in *Vacuolaria viridis*.

Further reports of *Gonyostomum semen* have been made by SMITH (5), who collected it in two sphagnum pools in West Yorkshire, England, and by CHADEFAUD (1), who described small mucus-producing bodies independent of the trichocysts in material collected by M. Lefèvre in a lake in Rambouillet Forest, 50 km. southwest of Paris. M. Lefèvre, in a letter dated July 2, 1936, informs us that this lake has a pH of 6.0–6.2 and an abundant aquatic flora of vascular plants, desmids, Protococcales, flagellates, diatoms, etc.

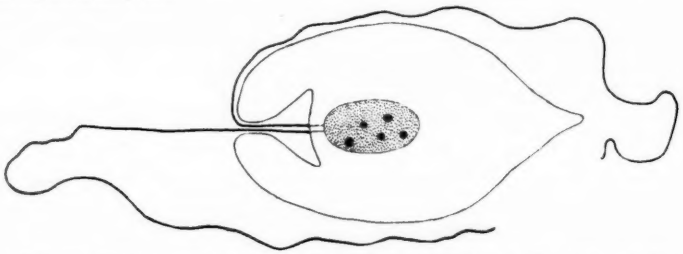

FIG. 1.—Diagrammatic dorsiventral longitudinal section of *Gonyostomum semen* from Woods Hole, Massachusetts, showing granular structure of nucleus and reconstruction of kinetic apparatus as seen in fixed and stained whole and sectioned material.

Specimens of *G. semen* taken from Cedar Swamp and from Iron Pond during 1935 and 1936 have been placed in the Herbarium of the Marine Biological Laboratory. We acknowledge gratefully the many courtesies shown to us by members of the staff of this Laboratory.—
FRANCIS DROUET and AARON COHEN, *Department of Botany, Marine Biological Laboratory.*

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CURRENT LITERATURE

The Cerro Cuadrado Petrified Forest. By G. R. WIELAND. Carnegie Institution of Washington. Publ. 449. 1935. Pp. 180.

The Cerro Cuadrado petrified forest of south-central Patagonia is described in great detail by WIELAND from sections of the seed cones, fragments of stem impressions, twigs, foliage, and basal portions of two seedlings. This South American material was collected by RIGGS in 1924 and is now among the exhibited collections of the Field Museum of Natural History. Descriptions of certain North American Araucarian cones are also included and discussed.

The subject is treated in seven chapters, the first of which is concerned with a discussion of the geologic age of the deposits. Unfortunately, WIELAND accepted the opinion of WINDHAUSEN that these remains represent early Triassic deposits, although he pointed out the possibility that they might belong to a post-Triassic age. In the most recent publication of the Argentine Survey[1] it is now said that the Cerro Cuadrado forest cannot be older than the Eocene. The more defined limits of the Cretaceous-Tertiary boundary have, however, been long subject to discussion.

The second chapter gives a taxonomic description of this collection of cones, represented by several dozen beautifully petrified specimens, together with other associated plant parts. WIELAND groups the cones into two generic types, *Proaraucaria* and *Pararaucaria*. Under *Proaraucaria mirabilis* he includes *Araucarites mirabilis* Spegazzini (1924), *A. windhauseni* Gothan (1925), and *Proaraucaria elongata* Wieland (1929), a series of 20 or more large spherical and ellipsoidal cones up to 9 cm. long and 6 cm. wide. They differ from living Araucarian cones in size and in the uniformly deep sulcus between the bract and seed scale. WIELAND mentions the evidence that the seeds may contain an endosperm and embryo, and this fact is established beyond doubt by a recent investigation by DARROW[2] on the same species.

DARROW states that the structure parallels so closely that of the scales of modern Araucarians, especially those of the Eutacta section, that *Proaraucaria mirabilis* furnishes no added evidence to settle the controversy centered about the status of the carpellate cone scales in *Araucaria*. However, it appears that DARROW did not show the cone scale of *P. mirabilis* in her diagrammatic sketch

[1] FREUQUELLI, JOAQUIN, Situación estratigraphica y edad de la zona con Araucarias al sul del curso inferior del Rio Deseado. Bol. Inform. Petrolif (Buenos Aires). No. 112. 1934.

[2] DARROW, BERTHA SCHWEITZER, A fossil Araucarian embryo from the Cerro Cuadrado of Patagonia. BOT. GAZ. **98**:328–337. 1936.

but the condition found in a modern *Araucaria*, without the conspicuous partial separation in the fused members so clearly shown in WIELAND'S figures and evident from an examination of this fossil material itself.

The stem impressions and foliage associated with these cones as well as parts of two seedlings belong to the Colymbea type of *Araucaria*, still represented in South America by the forests of *A. imbricata* found growing today on both flanks of the Andes about 600 miles farther north. A few of the *Proaraucaria* cones are treated as varieties of *P. mirabilis*. The fact that DARROW finds many Eutacta features in the cones serves to indicate that the two branches of the genus were not so sharply separated in past geologic time as they are in the living species.

Pararaucaria patagonica includes a collection of more cylindrical cones of smaller size, at least 5 cm. long and 2 cm. wide; somewhat larger than this if the missing eroded portions were restored. Like *Proaraucaria*, these forms are separated from living Araucarians by the much deeper separation of the cone scale and bract, and they differ from the former by the presence of important intermediate characters that would tend to unite them with *Pinus*, or place them closer to the Abietineae.

WIELAND includes the descriptions of several North American Araucarian cones, so that this monograph probably contains descriptions or photographs of all American material in which internal structure is preserved.

A chapter is devoted to the description of noted petrified forests found in all parts of the world, descriptions of log rafts and other conditions which may lead to petrifaction, followed by a chapter on the chemistry of petrifaction. Here the author brings together a discussion of some of the little known facts and theories bearing on petrifaction.

Chapter 5 is a treatment of cone and flower in which the inflorescence theory of the conifer seed cone and the ligular theory are contrasted and discussed. The former, sometimes spoken of as the brachyblast theory, is strongly favored as against the ligular theory. Of course there are many forms and variants of these theories which were not discussed, and WIELAND could scarcely review all that has been written in this century-old discussion of the morphology of the conifer cone within the space allotted. In his final chapter the author puts himself clearly on record as favoring the inflorescence theory. The Araucarian cone scale as shown by WIELAND'S sections appears to be much more than a ligule. Unfortunately, DOAK'S monograph[3] on pine needles and cones was still in press and could not be consulted, but it is significant that these two investigators, one working on the Araucariaceae and the other on the Pinaceae, have independently reached substantially the same conclusions. Both add many original observations on the morphology of cone development. DOAK regards the seed cones of the Pinaceae as compound structures, rep-

[3] DOAK, C. C., Evolution of foliar types, dwarf shoots, and cone scales of *Pinus*. Illinois Biol. Monographs 13, no. 3. 1935.

resenting a long-shoot axis with the secondary branches represented by the cone scales, while the pollen cones are regarded as simple cones borne in the axillary position of the dwarf branches.

There is a beautifully illustrated chapter describing the living Araucarian forests of South America and Australia, and a discussion of the former more worldwide distribution of *Araucaria*, now limited entirely to the southern hemisphere.

Whether a further investigation of the geologic age of these Araucarian fossils will actually prove them to be Triassic or Tertiary, this contribution has significance in demonstrating that this group possessed cone scale and bract which were actually more nearly separate and distinct in past geologic history and have become fused in modern forms, so that *Araucaria* may be derived from other coniferous stocks and need not be looked upon as a separate and distinct phyletic line.—J. T. BUCHHOLZ.

The Cultivated Races of Sorghum. By J. D. SNOWDEN. London: Adlard & Son, 1936. Pp. 274. Illustrated. 10*s.* 6*d.*

This valuable work is the most comprehensive yet published on the taxonomy of the genus *Sorghum*. It is the result of studies of panicles in the flowering and mature stages filed in the herbarium of the Royal Botanical Gardens at Kew, England. Most of the specimens were obtained from the British possessions, but all important sorghum producing countries, including the United States, are represented.

The book includes an extensive review of previous classifications, an elaborate bibliography of the classification and history of sorghums, numerous references to their culture and physiology, and an index listing nearly all of the Latin or botanical names and more than 1600 vernacular names that have been applied to varieties. The source of each variety is indicated. Historical, agricultural, and economic notes in addition to the keys and botanical descriptions are included for many varieties and species. The book concludes with a chapter on the relationships of the wild and cultivated sorghums.

The cultivated annual sorghums, often considered as one species, *Sorghum vulgare* Pers., are here classified into 31 species, 10 of which have been created by the author. He states: "There is a very wide range and much diversity in the shape and size of the fertile spikelets and grains, the texture of the glumes, and the relative size of the glumes and grains, and their relationship to one another. . . . . To sink such distinctive races of cultivated Sorghums all under one species is a decidedly retrograde step, which possesses no advantage whether from the standpoint of the practical cultivator, the geneticist, the cytologist, or the systematist . . . . the recognition of the most distinct races as species has the advantages of simplicity and directness. It also facilitates the preparation of a key to the cultivated sorghums which can be reasonably clear and not necessarily complicated. This is a task which is almost impossible of satisfactory

accomplishment under any system which attempts to crowd all these exceedingly numerous cultivated varieties into one vast unrestricted species."

Regardless of the advantages mentioned by the author, the separation of a group of varieties, having the same chromosome number and showing perfect interfertility among the hybrids, into a multiplicity of botanical species would seem to be questionable procedure in the light of modern genetics and cytology. Hybrid segregates from a single cross between two widely different sorghum varieties might easily fall into several of the author's so-called species, and possibly require the creation of additional species names in order to classify them.

The 31 species are subdivided into botanical varieties (subspecies) and these in turn into numbered forms. The cultivated varieties are listed in many cases without showing any distinctions between widely different varieties within a form. As an example, several distinct varieties of black-glumed kafir are listed under a single form and the very different group, hegari, is listed under the same form with only one of the numerous distinguishing characters separating hegari from the kafirs being indicated. In another form are listed three distinct American varieties, Dawn kafir, Reed kafir, and Chiltex, and several South African varieties with no attempt to distinguish between them. The reviewer is unable to determine the extent of such incomplete or erroneous groupings owing to insufficient familiarity with varieties not grown in America.

The studies were restricted to dried panicles without the opportunity of observing characters of other parts of the plants. Notes on plant characters were received from some experimenters and collectors sending specimens, but these were not usable generally because they were lacking for many other specimens. A classification of cultivated crop varieties, based upon morphological characters of herbarium specimens alone, of necessity leads to incongruous or artificial groupings that might be avoided by consideration of the characters of growing plants. Thus sweet and juicy-stemmed sorgos of the late maturing South African varieties, Honey and White African, and of the early, drought evading Chinese variety, Chinese amber, are included in the species *Sorghum dochna*, along with the broomcorns from southern Europe which have dry, non-sweet stems and very long panicle branches. Differences in seed size, and particularly in glume or seed color resulting from differences in environment, degree of maturity, or exposure to bleaching or weathering after maturity, have caused the author to place the same or very similar varieties in different subspecies. As an example, Dawn kafir is found as *S. caffrorum* var. *albofuscum* and again as var. *albidum*.

The major characters used to separate species, including the shape and size of the sessile spikelets, the thickness and degree of opening of the glumes, and the size of seeds, are all quantitative characters that frequently are difficult to classify correctly. Such characters as the persistence of glume hairs and pedicelled spikelets are subject to wide variation in dried material because of the shedding of these parts during maturity and handling.

Despite certain discrepancies, this book is an extremely useful reference work, and obviously represents an extensive and painstaking study of an immense collection of material.—J. H. MARTIN.

Production of Field Crops. By T. B. HUTCHESON, T. K. WOLFE, and M. S. KIPPS. New York: McGraw-Hill Book Company, 1936. Pp. xvii+445. figs. 110.

The present revision of a text now in use for more than a dozen years brings the subject matter more nearly up to date, as nearly as can be done in a text of this type. It has been somewhat shortened by the omission of some illustrations and tables; other new material has been added.

The general discussion chapters, as well as those dealing with specific crops, furnish interesting examples of the principles of ecology applied to crop plants. The distribution maps are clear cut, most of them based on data for 1929. Most of the illustrations have real point, but it seems unfortunate that agricultural texts should continue to use illustrations both inaccurate and misleading, as exemplified here by those of the corn grain and potato tuber.—E. J. KRAUS.

Encyclopedie Pratique du Naturaliste. XXX. Code Universel des Couleurs. By E. SEGUY. Paris: Paul Lechevalier. 1936.

A handy pocket-size folder contains 48 cardboard loose plates with 15 tints on each. The first color on each plate is a net color derived from blue, red, or yellow, which are the basic colors used. This initial color is progressively lightened to white in the first vertical column. Each shade thus obtained is toned down in a horizontal direction by the use of black, red, blue, yellow, or its own complement. Thus 720 different colors are derived.

Comparisons with various objects may be made direct, or by using masks of the color complementary to that of the shade isolated to obliterate the other tints on any particular card while a comparison is being made. Such masks are included in the set of cards. There is also a list of colors and a convenient index.—E. J. KRAUS.

The Biochemistry of the Lipids. By HENRY B. BULL. John Wiley and Sons. 1937. Pp. ix+169. Illustrated. $2.75.

This work is the successor to the mimeographed publication which appeared some months ago. The material has been in part rewritten, rearranged, and is better organized. There are nine chapters, besides the introductory one. These deal with the fatty acids; soaps; alcohols, waxes, and hydrocarbons; the sterols and related compounds; fats and oils; the phospholipids; cerebrosides; carbohydrate esters of the higher fatty acids; and emulsions. Author and subject indices are provided.—C. A. SHULL.